ANTIULCEROGENIC AND ULCER HEALING EFFECTS OF GALLIC ACID ALONE AND IN COMBINATION WITH H_2 BLOCKER FAMOTIDINE

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai
In partial fulfillment of the award of degree of

MASTER OF PHARMACY
(Pharmacology)

Submitted by

SAIKAT SEN

Under the joint supervision of

Dr. S. GOPALAKRISHNAN, B. Pharm., M.Sc (Pharm.), Ph.D.
Professor, Department of Pharmacology
&
Dr. K. ASOK KUMAR, M.Pharm., Ph.D.
Head, Department of Pharmacology

MARCH - 2008

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

Coimbatore - 641 044.

ANTIULCEROGENIC AND ULCER HEALING EFFECTS OF GALLIC ACID ALONE AND IN COMBINATION WITH H_2 BLOCKER FAMOTIDINE

Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University, Chennai
In partial fulfillment of the award of degree of

MASTER OF PHARMACY
(Pharmacology)

MARCH – 2008

COLLEGE OF PHARMACY

SRI RAMAKRISHNA INSTITUTE OF PARAMEDICAL SCIENCES

Coimbatore - 641 044.

Certificate

This is to certify that the dissertation entitled **"ANTIULCEROGENIC AND ULCER HEALING EFFECTS OF GALLIC ACID ALONE AND IN COMBINATION WITH H_2 BLOCKER FAMOTIDINE"** *was carried out by* ***SAIKAT SEN*** *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my direct supervision and guidance to my fullest satisfaction.*

Dr. S. Gopalakrishnan, B.Pharm., M.Sc., Ph.D.
Professor, Department of Pharmacology,
College of Pharmacy, SRIPMS,
Coimbatore – 44.

Place: Coimbatore
Date:

Certificate

This is to certify that dissertation entitled **"ANTIULCEROGENIC AND ULCER HEALING EFFECTS OF GALLIC ACID ALONE AND IN COMBINATION WITH H_2 BLOCKER FAMOTIDINE"** *was carried out by* ***SAIKAT SEN*** *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the joint supervision of* ***Dr. S.Gopalakrishnan, B.Pharm., M.sc (Pharm.), Ph.D****. and* ***Dr. K. Asok Kumar, M.Pharm., Ph.D.*** *Head, Department of Pharmacology, College of Pharmacy, SRIPMS, Coimbatore-44.*

Dr. T. K. Ravi, M.Pharm., Ph.D. FAGE.
Principal,
College of Pharmacy, SRIPMS,
Coimbatore – 44.

Place: Coimbatore
Date:

Certificate

This is to certify that the dissertation entitled **"ANTIULCEROGENIC AND ULCER HEALING EFFECTS OF GALLIC ACID ALONE AND IN COMBINATION WITH H_2 BLOCKER FAMOTIDINE"** *was carried out by* ***SAIKAT SEN*** *in the Department of Pharmacology, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under my co-guidance and supervision to my fullest satisfaction.*

Dr. K. Asok Kumar, ***M.Pharm., Ph.D.***
Head of the Department,
Department of Pharmacology,
College of Pharmacy, SRIPMS,
Coimbatore - 44 .

Place: Coimbatore
Date :

ACKNOWLEDGEMENT

It is my pride and prejudice to express my heartfelt thanks and deep sense of gratitude to my esteemed guide ***Dr. S. Gopalakrishnan****, B.pharm., M.sc(pharm.,), Ph.d., Professor, Department of Pharmacology, for his invaluable and unique guidance that he had given me throughout the course of my thesis work.*

I am concerted to express my profound gratitude to my co-guide ***Dr. K. Asok Kumar****, M.Pharm., Ph.D., Head, Department of Pharmacology, under whose innovative ideas, constant inspiration, help and encouragement my work has been carried out.*

*To my beloved Principal****, Dr. T. K. Ravi****, M.Pharm., Ph.D. FAGE, College of Pharmacy, SRIPMS, Coimbatore, I wish to express my sincere thanks and gratitude for his whole hearted co-operation in rendering the facilities to proceed with this study.*

I may also thank ***Mrs. M. Uma Maheswari****, M.Pharm., (Ph.D), Assistant Professor,* ***Mr. A. T. Sivashanmugam,*** *M.Pharm and* ***Mrs. V. Subhadra Devi****, M.Pharm Lecturers, Department of Pharmacology for their encouragement and moral support that kept me poised throughout the tenure of my thesis work.*

*I would like to express my sincere thanks to **Mr. M. Gopal Rao**, Vice Principal, Head, Department of Pharmaceutics, **Mrs. M. Gandhimathi**, Assistant Professor, Department of Pharmaceutical Analysis and **Mr. S. Krishnan**, Head, Department of Pharmaceutical Biotechnology, for their support in providing the facilities to carry out the work.*

*I am also extending sincere thanks to **Mr. H. John** and **Mr. A. Ramakrishnan** Lab Assistants, **Mrs. M. Hamsaveni and Mrs. K. Latha**, **Mr. S. Muruganantham** Lab attenders and **Mrs. R. Vatsala**, Storekeeper. My special thanks goes to the **library staff** for providing library facilities.*

*I extend my deep sense of gratitude to my friends **Remya Raju, Neethu Michel, N. Umamageswari, R. Chandra, Kaladhar P., S. S. Somanathan, Bahrudeen A., P. Bharathi, Sham, Ashim Sen, Sambit Deb, Devraj Pal, Avijit Das, M. Raja Omar Sheriff, J. Yuvaraju, Rakesh Chawla** for their valuable suggestions, co-operations and encouragement and a special word of thanks my best friend **Raja Chakraborty** for his valuable help and continuous support throughout.*

*I am thankful to **M/s Saraswati Computer Center** who has helped me to make the dissertation documented in a beautiful way.*

*I remain greatly indebted to my beloved **parents, my family** and all **my teachers** for their precious love, affection, prayer and moral support which guided me in right path and are also the backbone for all my successful endeavors in my life.*

*I express my sincere thanks and respectful regard to our managing trustee Sevaratna **Dr. R. Venkatesalu Naidu** for all the facilities, which were provided to me in this institution.*

*Above all, allow me to proclaim the over powering presence of **The Almighty** who is the source of all wisdom and knowledge for the successful completion of this dissertation.*

Saikat Sen

Dedicated to My Beloved Parents

LIST OF TABLES

CONTENTS

LIST OF ABBREVIATIONS

ACh	- Acetylcholine
ALE	- *Anogeissus latifoloa*
ATP	- Adenosine triphosphate
b.w.	- Body weight
BSA	- Bovine serum albumin
cAMP	- Cyclic 3´, 5´ - adenosine monophosphate
CAT	- Catalase
CCK_2	- Cholecystokinin receptor
cNOS	- constitutive nitric oxide synthase
COX	- Cyclooxygenase
CRS	- Cold resistant stress
DMSO	- Dimethylsulphoxide
DNA	- Deoxyribo nucleic acid
dNTP	- Deoxynucleoside triphosphate
DPPH	- 2,2-diphenyl-1-picryl hydrazylhydrate
DTNB	- 5, 5, Dithiobis (2 - nitro benzoic acid)
ECL	- Enterochromaffin like cell
Enz	- Enzyme
Fe-NTA	- Ferric nitrilotriacetic acid
FM	- Famotidine
G6PD	- Glucose 6-phosphate dehydrogenase
GA	- Gallic acid
GPCR	- Guanyl protein coupled receptor

GPx	- Glutathione peroxidase
GR	- Glutathione reductase
GSH	- Reduced glutathione
H_2O_2	- Hydrogen peroxide
HFD	- High fat diet
HIST	- Histamine
HOCl	- Hypochlorus acid
HP	- Hydroperoxides
HPTLC	- High performance thin layer chromatography
IFN-β	- Beta interferone
IgE	- Immunoglobulin
iNOS	- Inducible nitric oxide synthase
JGLE	- *Jasminum grandiflorum*
LPO	- Lipid peroxidation
M	- Muscarinic receptor
MDA	- Malondialdehyde
MGSTI	- Microsomal glutathione S-transferase
min	- Minute
MPx	- Myeloperoxidase
N	- Nicotinic receptor
NADP	- Nicotinamide adenine dinucleotide phosphate
NADPH	- Nicotinamide adenine dinucleotide phosphate reduced
nm	- Nanometer
nM	- Nanomole
NPSH	- Nonprotein thiol compound

NSAIDs	- Non steroidal antiinflammatory drugs
PAF	- Platelet activating factor
PG	- Prostaglandin
PGE_2	- Prostaglandin E_2 receptor
PL	- Pylorus ligation
ROS	- Reactive oxygen species
rpm	- Rotation per minute
s.c.	- Subcutaneous
SOD	- Superoxide dismutase
TAG	- Triacylglycerol
TBA	- Thiobarbituric acid
TBARS	- Thiobarbituric acid reactive substances
TCA	- Trichloro acetic acid
UA	- Usnic acid
µg/ml	- Microgram per milliliter
µM	- Micromole
µl	- Micro liter

1. INTRODUCTION

1.1 STOMACH

The gastrointestinal tract is one of the major exocrine systems in the body. Stomach is a J- shaped most dilated part of the alimentary canal, situated between oesophagus and small intestine, which reserves food and helps in digestion. It is situated directly under the diaphragm in the epigastric, umbilical and left hypochondriac regions of the abdominal cavity. The stomach is continuous with the esophagus at the cardiac sphincter and with the duodenum at the pyloric sphincter. In the stomach, digestion of starch continues, digestion of protein and triglycerides begins, the semisolid bolus converts to liquid and certain substances are absorbed (Ghosh, 2007; Waugh and Grant, 2006).

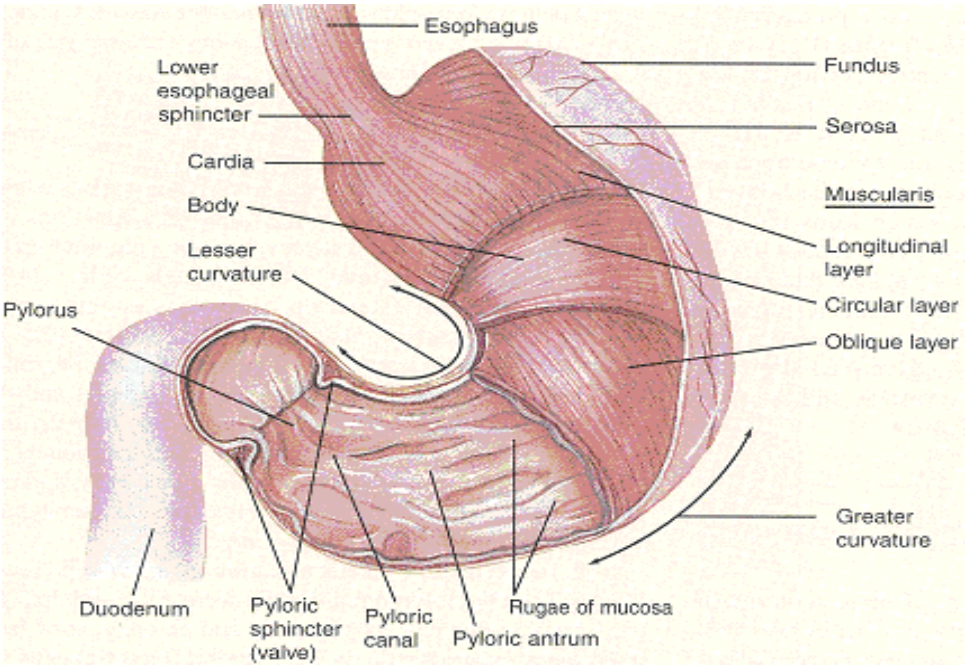


Fig 1: Anterior view of stomach (Tortora and Derrickson, 2006)

1.1.1 Anatomy of stomach

The stomach is divided into four regions –

Cardia : It surrounds the superior opening of the stomach.

Fundus : The left portion of the cardia is fundus.

Body : It is large central portion of the stomach, inferior to the fundus.

Pyloric part : It is the region of stomach that connects to the duodenum. It is divided into two parts

i. Pyloric antrum – connects to the body of stomach.

ii. Pyloric canal – leads into duodenum.

The pylorus joined with duodenum via a sphincter called pyloric sphincter, which guards the opening between stomach and duodenum. When the stomach is inactive the pyloric sphincter is relaxed and open, and when the stomach contains food the spincter is closed. The concave medial border of the stomach is called the lesser curvature and the convex lateral border is called the greater curvature (Tortora and Derrickson, 2006).

1.1.2 Histology of stomach

The wall of stomach consists of four usual layers

Gastric mucosa – The surface of the mucosa is a layer of simple columnar epithelial cells called surface mucosa cells. It contains areolar connective tissue called lamina propria and smooth muscle called muscularis mucosae. The epithelial cells extend down into the lamina propria, when they form columns of gastric secretary cells

called gastric glands, which line many narrow channels called gastric pits. The gastric glands have three types of exocrine glands –

a) Mucous neck cells – secretes mucus

b) Chief cells – secretes pepsinogen and gastric lipase

c) Perietal cells – produce intrinsic factor and hydrochloric acid

In addition, gastric glands include a type of enteroendocrine cell called G cell, which is located mainly in the pyloric antrum and secretes the hormone gastrin into blood stream.

Submucosa – the submucosal layer of stomach composed of aerolar tissue.

Muscularis external – It consists of 3 layers

i. Outer – Longitudinal layer

ii. Middle – Circular layer

iii. Inner – Oblique layer

Serosa – Serosa composed of simple squamous epithelial cell called mesothelium and areolar connective tissue. Serosa covers the visceral peritoneum portion of the stomach (Tortora and Derrickson, 2006).

1.1.3 Functions of stomach

Stomach stores the food temporarily so that digestive enzymes and pepsin can act on the food for digestion. Mechanical breakdown of food by all the three smooth muscles enable the stomach to act as a churn. Stomach also regulates the passage of gastric contents into the duodenum. When the chyme is sufficiently acidified and liquefied, the pyloric antrum forces small jets of gastric contents through the pyloric sphincter into the duodenum. This sphincter is normally closed,

preventing backflow of chyme into stomach. The motility and secretion are regulated by parasympathetic nerve stimulation. The chemical digestion also takes place in stomach like pepsin converts protein to polypeptides. Limited absorption of water, alcohol and some lipid soluble drugs occur from stomach. Hydrochloric acid (HCl) present in the gastric juice kills the bacteria and other harmful substances and provide non specific defense against microbes. The intrinsic factor secreted in stomach is needed for absorption of vitamin B_{12}. Stomach also secretes gastrin hormone. Stomach provides the acidic environment which is required before absorption of iron (Waugh and Grant, 2006; Chatterjee, 1985).

1.1.4 Gastric juice and its function

About 2-3 liters of gastric juice secreted daily by specialized secretary glands in the mucosa. The stomach secretes gastric juice and it contains –

- **Mineral salts and water** – liquefies the swallowed food.
- **Mucus** – prevent mechanical and chemical injury to the stomach by lubricating the contents and by acting as a barrier between stomach and corrosive gastric juice.
- **Hydrochloric acid** – denatures the proteins (unfold) present in food partially, acidify the food and stops the action of salivary amylase, kills ingested microbes, provide acid environment needed for digestion by pepsinogen.
- **Pepsin –** only proteolytic enzyme in the stomach. Pepsin is secreted in an inactive form called pepsinogen in the stomach.

Pepsinogens activate the pepsin by hydrochloric acid and by pepsin already present in stomach. They begin the digestion of protein by breaking them into smaller fragments. It acts at pH 1.5 to 3.5.

- **Intrinsic factor (a protein)** – necessary for the absorption of vitamin B_{12}.
- **Gastric lipase** – splits the short-chain triglycerides in fat molecules into fatty acid and monoglycerides. Stomach also secretes gastrin into blood (Waugh and Grant, 2006).

1.1.5 Phases of gastric secretion

The secretion of gastric juice occurs by 3 phase named cephalic, gastric and intestinal phase. The digestive activities also occur in this three overlapping phases.

Cephalic phases

This flow of gastric juice occurs before food reaches the stomach. Smell, sight, thought or taste of food activates neural centers in the cerebral cortex, hypothalamus and brain stem.

Gastric phase

The hormonal and neural mechanisms regulate the gastric phase of digestion. Hormonal regulation is stimulated by the presence of food the enteroendocrine cells in the pyloric antrum and duodenum secretes gastrin. Gastrin, circulating blood which supplies the stomach, stimulates the gastric glands to produce more gastric juice. Gastrin secretion is suppressed when the pH in the pyloric antrum falls to 1.5. Under neural mechanism, when stomach walls are distended or pH increases, the stretch receptors and chemoreceptors are activated.

From the stretch receptor and chemoreceptor, nerve impulses propagate to the submucosal plexus, where they activate parasympathetic and enteric neurons. The resulting nerve impulses cause waves of peristalsis and continue to stimulate the flow of gastric juice.

Intestinal phase

This phase starts when partially digested food contents enter the small intestine from stomach. The two hormones secretin and cholecystokinin are produced by endocrine cells in intestinal mucosa and they slow down the secretion of gastric juice and reduce gastric motility. By slowing the emptying rate of the stomach, the contents of the duodenum become more thoroughly mixed with bile and pancreatic juice. This phase of gastric secretion is most marked following meal with high fat content (Waugh and Grant, 2006; Tortora and Derrickson, 2006).

1.1.6 Physiology of gastric secretion

Gastric acid secretion is a complex, continuous process in which multiple central and peripheral factors contribute to a common endpoint: the secretion of H^+ by parietal cells. The regulation of acid secretion by parietal cells is especially important in peptic ulcer and constitutes a particular target for drug action. The secretion of the parietal cells is an isotonic solution of hydrochloric acid (150 mmol/l). The Cl^- is actively transported into canaliculi in the cell which communicate with the lumen of the gastric glands and thus with lumen. The Cl^- secretion is accompanied by K^+, which is then

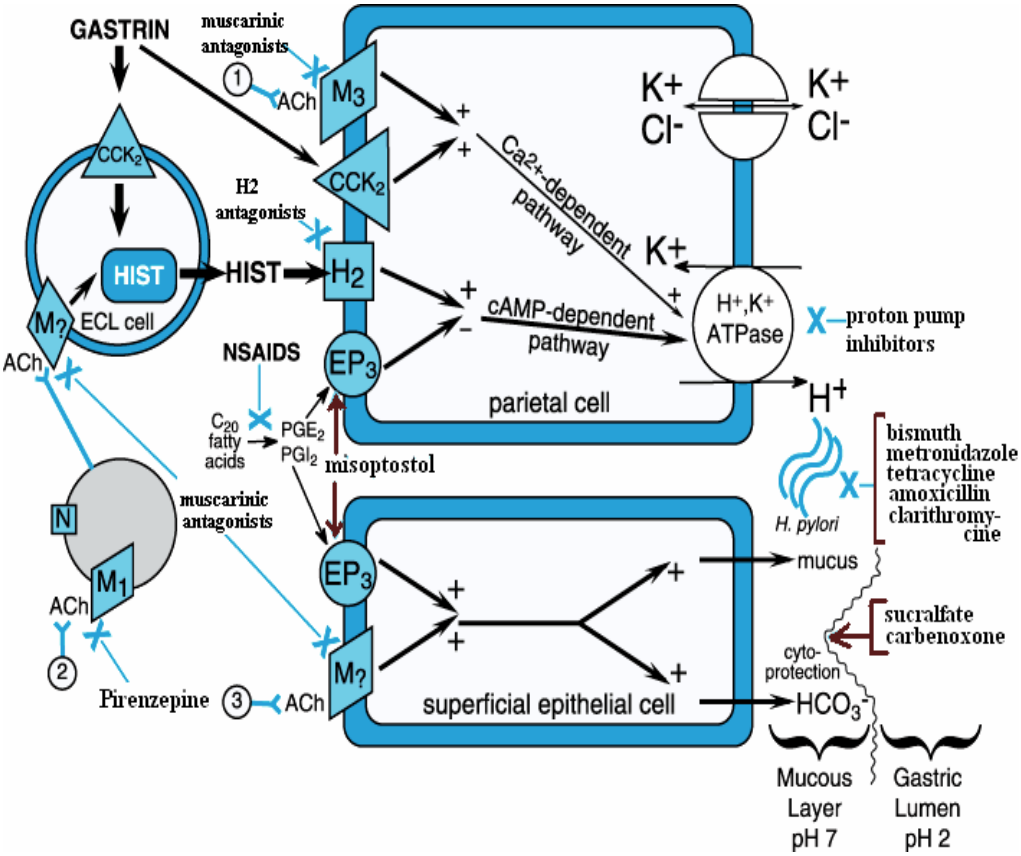
exchanged for H^+ from within the cell by a H^+, K^+ - ATPase. Carbonic anhydrase catalyses the combination of carbondioxide and water to give carbonic acid (HCO_3^-), which dissociate into H^+ and bicarbonate ions (Rang *et al*., 2003; Bevan and Thompson, 1983).

$$H_2O + CO_2 \xrightarrow{\text{carbonic anhydrase}} H^+ + HCO_3^-$$

Secretion of gastric acid is accomplished by activity of an ion-motive ATPase that exchanges cytosolic H^+ for luminal K^+, resulting in acidification of extacellular surface of parietal cell and secretion of gastric juice. Parietal cell H^+, K^+ - ATPase, often designated the gastric ‘‘proton pump’’, derives its energy for transport of H^+ and K^+ from the hydrolysis of adenosine tri phosphate (ATP). In the parietal cell, the enzyme has access to the canalicular membrane that provides a pathway for inward K^+ transport in exchanging for outer H^+ transport. The transport activity of H^+, K^+-ATPase is regulated by levels of intracellular second messengers, either cAMP or calcium ion (Munson, 1995).

Neuronal (acetylcholine, ACh), paracrine (histamine), and endocrine (gastrin) factors all regulate acid secretion. Their specific receptors (M_3, H_2, and CCK_2 receptors, respectively) are on the basolateral membrane of parietal cells in the body and fundus of the stomach. The histamine (H_2) receptor is a G-protein coupled receptor (GPCR) that activates the G_s-adenylylcyclase-cyclic AMP-PKA pathway. ACh and gastrin signal through GPCRs that couple to the G_q-PLC-IP_3-Ca^{2+} pathway in parietal cells. In parietal cells, the cyclic

AMP and the Ca^{2+}-dependent pathways activate H^+,K^+-ATPase (the proton pump), which exchanges hydrogen and potassium ions across the parietal cell membrane. This pump generates the largest known ion gradient in vertebrates, with an intracellular pH of about 7.3 and an intracanalicular pH of about 0.8 (Brunton *et al*., 2006).



Acetylcholine (ACh), muscarinic (M), and nicotinic (N) receptors; gastrin, cholecystokinin receptor (CCK_2); histamine (HIST), H_2 receptor; and prostaglandin E_2 receptor (EP_3). The X indicates antagonism effect. ECL cell are enterochromaffin-like cell. The stimulatory effect (+) or inhibitory (-). 1 and 3 indicate possible inputs from postganglionic cholinergic fibers, while 2 shows neural input from the vagus nerve.

Fig 2: Regulation of acid secretion by parietal cells and the site of action of drugs (Brunton *et al*., 2006)

1.1.7 Gastric defense system

A number of mechanisms adopted by the stomach to protect itself from acid induced damage. An adequate mucosal blood flow, oxygen requirements of the gastric mucosa is needed to protect the mucosa. Gastric mucus is an important factor for the gastric mucosa and consists of viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoprotein which cover the gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant and thus can reduce mucosal damage mediated by oxygen free radicals. The secretion of a mucus layer protects gastric epithelial cells. Gastric mucus secreted in soluble form but quickly forms an insoluble gel that coats the mucosal surface of the stomach, slows ion diffusion, and prevents mucosal damage by macromolecules such as pepsin (Repetto and Llesuy, 2002).

Secretion of mucus is stimulated by prostaglandins E_2 and I_2. Prostaglandins also directly inhibit gastric acid secretion by interacting with parietal cells, EP_3 receptor which are negatively coupled to adenylyl cyclase by means of Gi protein. Prostaglandins inhibit the formation of cAMP and reduce the activity of H^+,K^+-ATPase. Prostaglandin also enhances mucosal blood flow. An important part of the normal mucosal defense is the secretion of bicarbonate ions by superficial gastric epithelial cells which neutralizes the acid in the region of the mucosal cells, thereby raising pH and preventing acid-

mediated damage. Continued renewal of the mucosal cell layer is an important part of the defense system. Somatostatin also inhibits gastric secretion but the release of somastostatin is inhibited by acetylcholine. Nitricoxide also participates in the gastric defense mechanism. It increases the synthesis of prostaglandins in physiological and pathological conditions and influences the gastric mucosal blood flow and gastric mucus secretion (Gurbuz and Yesilada, 2007; Brunton *et al.,* 2006; Clark *et al.,* 1992).

1.2 PEPTIC ULCER

Peptic ulcer disease is characterized by inflamed lesions or excavations of the mucosa and underlying tissue of the upper gastrointestinal tract. The ulcers are the result of damage to the mucus membrane that normally protects the esophagus, stomach and duodenum from gastric acid and pepsin (Brenner and Stevens, 2006). The pathophysiology of this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin (Brunton *et al.,* 2006; Goel and Sairam, 2002). Gastric ulcer is often a chronic disease and may persist for 10-12 years characterized by repeated episode of healing and re-exacerbations (Rao *et al*., 2004).

1.2.1 Epidemiology

Peptic ulcer is the most common, chronic gastrointestinal disorder and has become a common global health problem affecting a large number of people world wide and still a major cause of

morbidity and mortality (Chan and Leung, 2002). Geographically, the disease is prevalent throughout the world, in USA annually 3.7 million people are affected by this disease.

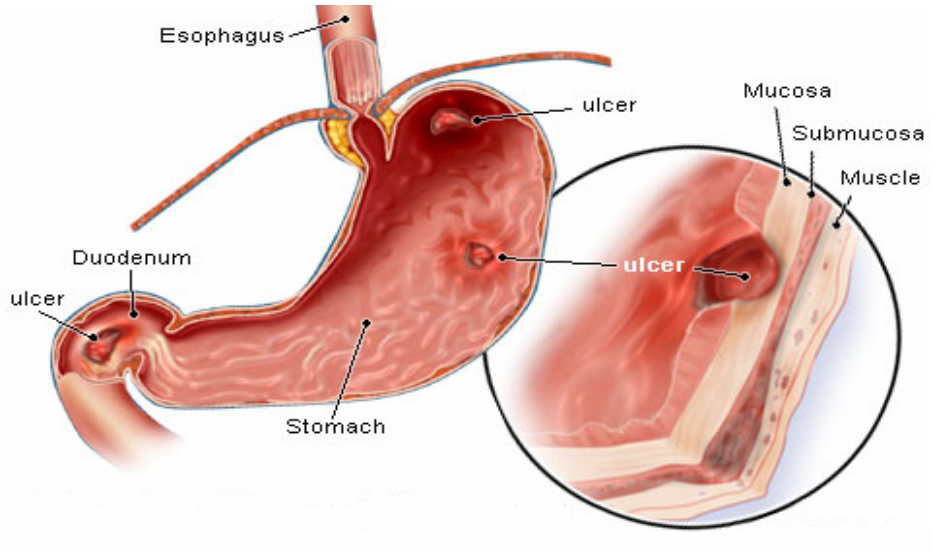


Fig 3: Site of peptic ulcer in different parts of the stomach

1.2.2 Factors causing peptic ulceration

Gastric injury induced by NSAIDs

Non steroidal anti-inflammatory drugs (NSAIDs) are used widely in the treatment of pain, fever, inflammation, rheumatic and cardiovascular disease, and more recently for the prevention of colon cancer and Alzheimer's disease. Chronic administration of these drugs is often associated with the development of adverse gastrointestinal effects, such as gastric erosions, gastric and duodenal ulceration and severe complications such as gastrointestinal hemorrhage and perforation. NSAIDs like aspirin, indomethacin is known to induce ulcer by inhibition of cyclooxygenase enzyme (COX) and suppression

of prostaglandin (PG) mediated effects on mucosal protection. Endogenous prostaglandins regulate the mucosal blood flow, epithelial cell proliferation, epithelial reconstitution, mucosal immunocyte function, mucus and bicarbonate secretion. Beside, these neutrophil and oxygen radical-dependant microvuscular injuries may be important processes that lead to mucosal damage in response to NSAIDs administration. These agents cause the activation of neutrophils and their adherence to the vascular endothelium, hence blocking capillaries and reducing local gastric blood flow. It also reduces the hydrophobicity of the mucus gel layer by changing the action of surface-active phospholipids. These drugs cause an increase in acid and pepsinogen secretion (Hiruma-Lima *et al*., 2006; Berenguer *et al*., 2006; Toma *et al.,* 2005; Jainu and Devi, 2004; Blandizzi *et al*., 2005; Chang and Leung, 2002).

Helicobacter pylori

Helicobacter pylori, a gram-negative rod, has been associated with gastritis and subsequent development of gastric and duodenal ulcers, gastric adenocarcinoma and gastric B-cell lymphoma (Brunton *et al.,* 2006). Gastroduodenal diseases caused by *H. pylori* are associated with infiltration of gastric mucosa by neutrophil, lymphocyte, monocytes and plasma cell (Gooz *et al*., 2001)

Stress

Stress plays an important role in etiopathology of gastro-duodenal ulceration. Increase in gastric motility, vagal over activity; mast cell degranulation; decreased gastric mucosal blood flow; and

decreased prostaglandin synthesis; are involved in genesis of stress induced ulcers (Rao *et al*., 2004). Gastric stress ulceration is probably mediated by release of histamine with enhanced acid secretion and reduced mucus production (Toma *et al*., 2005).

Smoking

Cigarette smoking can also increase the chance of getting an ulcer. Smoking also slows the healing of existing ulcer and contributes to ulcer reoccurrence (Clark *et al*., 1992; Walker and Taylor, 1979).

Blood group

The incident of peptic ulcer is higher in the people having blood group O than those of blood groups A and B (Nelson and Cox, 2005; Bowman and Rand, 1980).

Sex

Duodenal ulcer more commonly seen in man than in women, though gastric ulcer occurs to an equal extent in man and women (Bowman and Rand, 1980).

Dietary factors

Caffeine

The caffeine can stimulate acid secretion in stomach and can aggravate an existing ulcer.

Alcohol

There is a little evidence of an association between alcohol use and peptic ulcer. Ulcers are common in people who have cirrhosis of the liver, a disease often linked to heavy alcohol consumption.

Dietary salt

The evidence was found between an association of gastric ulcer and dietary salts. The occurrence of gastric ulcer may be linked to the amount of dietary salt consumption. The salt was shown to induce gastritis of experimental animals (Duggan and Duggan, 2006; Sonnenberg, 1986).

1.2.3 Treatment of peptic ulcer

Drugs employed in the treatment of peptic ulcer have three therapeutic aims (i) to relieve pain (ii) to accelerate healing and (iii) to prevent ulcer recurrence. Therefore three therapeutic approaches are to reduce aggressive forces by lowering H^+ output, to increase protective forces by means of mucoprotectants and to eradicate *H. pylori* (Tripathi, 2003; Lullmann *et al.*, 2000).

Drugs used in the treatment of peptic ulcer

1. Reduction of gastric acid secretion

a) Proton pump inhibitors - Omeprazole, Pentoprazole

b) H_2 antihistamines - Ranitinide, Cimetidine, Famotidine

c) Anticholinergics - Pirenzepine, Propantheline

d) Prostaglandin analogues - Misoprostol, Enprostil, Rioprostil

2. Neutralization of gastric acid (Antacids)

a) Systemic - Sodium bicarbonate, Sodium citrate

b) Non systemic - Magnesium hydroxide, Magnesium trisilicate, Aluminium hydroxide gel, Calcium carbonate.

3. Ulcer protective agent

Sucralfate, Colloidal bismuth subcitrate

4. Ulcer healing drugs or cytoprotective agents

Carbenoxolone, Rebemipide

5. Anti - *H. pylori* drugs

Amoxicillin, Clarithromycin, Metronidazole, Tinidazole, Tetracycline.

1.2.4 Induction of experimental ulcer

Antiulcer activity can be evaluated in rats by employing the following methods (Parmar and Desai, 1993). They are broadly divided into 3 types–

A) Gastric antiulcer study

a) Pylorus ligated ulcer (Shay rats)

b) NSAIDs induced gastric damage - Aspirin, Indomethacin, Phenylbutazone generally used to induce ulcer in rats.

c) Stress ulcer – like Restraint ulcers, water immersion-induced restraint ulcer, cold and restraint ulcers, swimming stress ulcers, activity stress ulcer.

d) Reserpine induced ulcer (chronic ulcer model)

e) Acetic acid induced chronic ulcer (chronic ulcer model)

f) Serotonin induced ulcer

g) Dimapit induced gastric ulcer

h) Endotoxin (Lipopolysaccharide B) induced gastric ulcer

i) Aspirin plus pylorus ligated ulcer - This is one of the useful and popular ulcer models. Aspirin like NSAIDs are one of the important factors which cause the ulcer in human and used in

animals to produce ulcer experimentally. Pylorus ligation also one of the important experimental ulcer model where increased amount of acid and pepsin causes ulcer. When aspirin is administered to pylorus ligated rats, it further aggravated the acidity and the resistance of the gastric mucosa was decreased thereby imposing extensive damage to the glandular region of the stomach (Sanmugapriya and Venkataraman, 2007).

B) Gastric cytoprotection study

The following methods are employed to produce mucosal injury in experimental animals and evaluate cytoprotective effect of drug.

a) 30 mg of aspirin suspended in 0.15 M HCl

b) Absolute ethanol

c) 0.6 M hydrochloric acid

d) 25 % sodium chloride

e) 80 mM of sodium taurocholate

f) Boiling water (thermal injury)

C) Duodenal anti-ulcer study

Duodenal ulcer can be induced by cysteamine, dulcerozine, dimaprit, 1–methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP), indomethacin plus histamine and through the subcutaneous (s.c) infusion of pentagastrin and carbachol.

1.2.5 Effect of oxidative stress, free radicals in peptic ulcer

Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including neurodegenerative disorder, atherosclerosis, ischemic heart disease, ageing process,

inflammation, diabetes, immunodepression, neurodegenerative condition and also peptic ulcer (Bafna and Balaraman, 2005; Balaraman *et al*., 2004). ROS caused gastric damage by physical, chemical, psychological factors that lead to gastric ulceration in human and experimental animals (Rao *et al*., 2004)

Oxidative stress plays an important role in the pathogenesis of gastric ulcer. Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels (Tian *et al*., 2007). Reactive oxygen metabolites include true free radicals and other toxic compounds derived from oxygen metabolism, including hydrogen peroxide and hypochlorite (Davies and Rampton, 1994). The ROS formed from several sources like mitochondrial cytochrome oxidase, xanthine oxidases, neutrophils and transitional metals. The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells may contribute to gastric mucosal damage. Oxygen free radicals are detrimental to the integrity of biological tissue and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism. The damage to membrane proteins decreases membrane permeability, activities of enzymes and receptors and activation of cells (Sannomiya *et al.,* 2005; Demir *et al.,* 2003; Repetto and Llesuy, 2002; Ali *et al*., 1996). ROS play important role

in formation of gastric ulcers induced by stress, ethanol, NSAIDS, pylorus ligation and *H. pylori* infection. (Halici *et al*., 2005; Bafna and Balaraman, 2005).

Reactive oxygen species includes – Radicals such as superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$) and non radicals like hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1\Delta g$), peroxy nitrate ($ONOO^-$).

Superoxide anions ($O_2^{\bullet -}$) is one of the important ROS, which is attributed in tissue and gastric damage and responsible for lipid peroxidation. Superoxide radicals also have the capability to decrease the activity of other antioxidant defense system enzyme such as catalase and glutathione peroxidase (GPx). Superoxide anions also cause damage to the ribonucleotide which is required for DNA synthesis. The protonated form of $O_2^{\bullet -}$ is $HO_2^{\bullet}$, which is more reactive and able to cross the membrane and causes damage to tissue.

Hydrogen peroxide (H_2O_2) is not a radical but it produces toxicity to cell by causing DNA damage, membrane disruption and release calcium ions within cell, resulting in calcium dependent proteolytic enzyme to be activated.

Hydroxyl ($OH^{\bullet}$) radical is most reactive chemical species. It is a potent cytotoxic agent and able to attack and damage almost every molecule found in living tissue. One $OH^{\bullet}$ can result in conversion of many fatty acid side chains into lipid hydroxyl peroxides.

Hypoclorous acid (HOCl) is produced by the enzyme myeloperoxidase in activated neutrophils and initiates the deactivation

of antiproteases and activation of latent proteases leading to tissue damage. It has ability to damage biomolecules, directly and also decomposes to liberate toxic chlorine (Halliwell and Gutteridge, 1999).

1.2.6 Role of antioxidant defense system in peptic ulcer

The body has developed several endogeneous antioxidant defence systems to deal with the production of ROS. The antioxidant enzymes play a major role in cell defense against acute oxygen toxicity. Their main function is to protect membrane and cytosolic components against damage caused by free radicals. This system is divided into two groups viz., enzymatic and non enzymatic. The enzymatic defense system include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and the non enzymatic defense system included vitamin E, vitamin A, vitamin C and reduced glutathione (GSH) (Demir *et al.*, 2003, Harris, 1992).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is an important endogenous antioxidant enzyme act as the first line defense system against ROS which scavenges superoxide radicals. SOD catalyzes the dismutation of superoxide ($O_2^{\bullet -}$) to H_2O_2 and O_2.

$$O_2^{\bullet} + O_2^{\bullet} + 2H^{+} \longrightarrow H_2O_2 + O_2$$

Glutathione peroxidase (GPx)

Glutathione peroxidase present in the cytoplasm of the cells. It

consists of four protein subunits; each of which contains one atom of the element selenium at its active site. GPx remove H_2O_2 by coupling its reduction to H_2O with oxidation of reduced glutathione (GSH). Thus it protects cell against oxidative injury caused by H_2O_2 and prevent the formation of hydroxyl radical from H_2O_2 (Halliwell and Gutteridge, 1999).

$$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$$

GPx catalyse GSH dependent reduction of fatty acid hydroperoxides (eg, linoleic acid, cholesterol 7β - hydroperoxide) and various synthetic hydroperoxide. But it can not act upon fatty acid peroxides esterified to lipid molecule in lipoprotein or membrane.

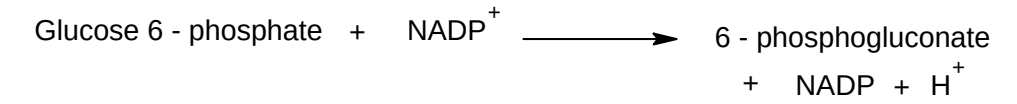
Glutathione reductase (GR)

The ratio of reduced to oxidized glutathione (GSH/GSSG) in normal cell is high so that there must be mechanism for reducing GSSG back to GSH. The reduction of GSSH to GSH is achieved by the enzyme glutathione reductase (GR) in the presence of NADPH.

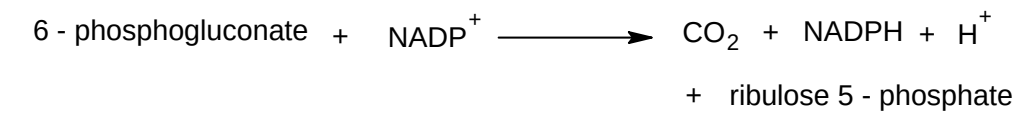
$$GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP^+$$

The NADPH required is provided in both animal and plant tissue by several enzyme systems, the most important one is the oxidative pentose phosphate pathway. Glucose-6-phosphate dehydrogenase is the first enzyme in this pathway.

The rate at which the pentose phosphate pathway operates is controlled by the supply of $NADP^+$ to Glucose-6-phosphate dehydrogenase (G6PD) (Halliwell and Gutteridge, 1999).



Followed by 6 - phosphogluconate dehydrogenase,



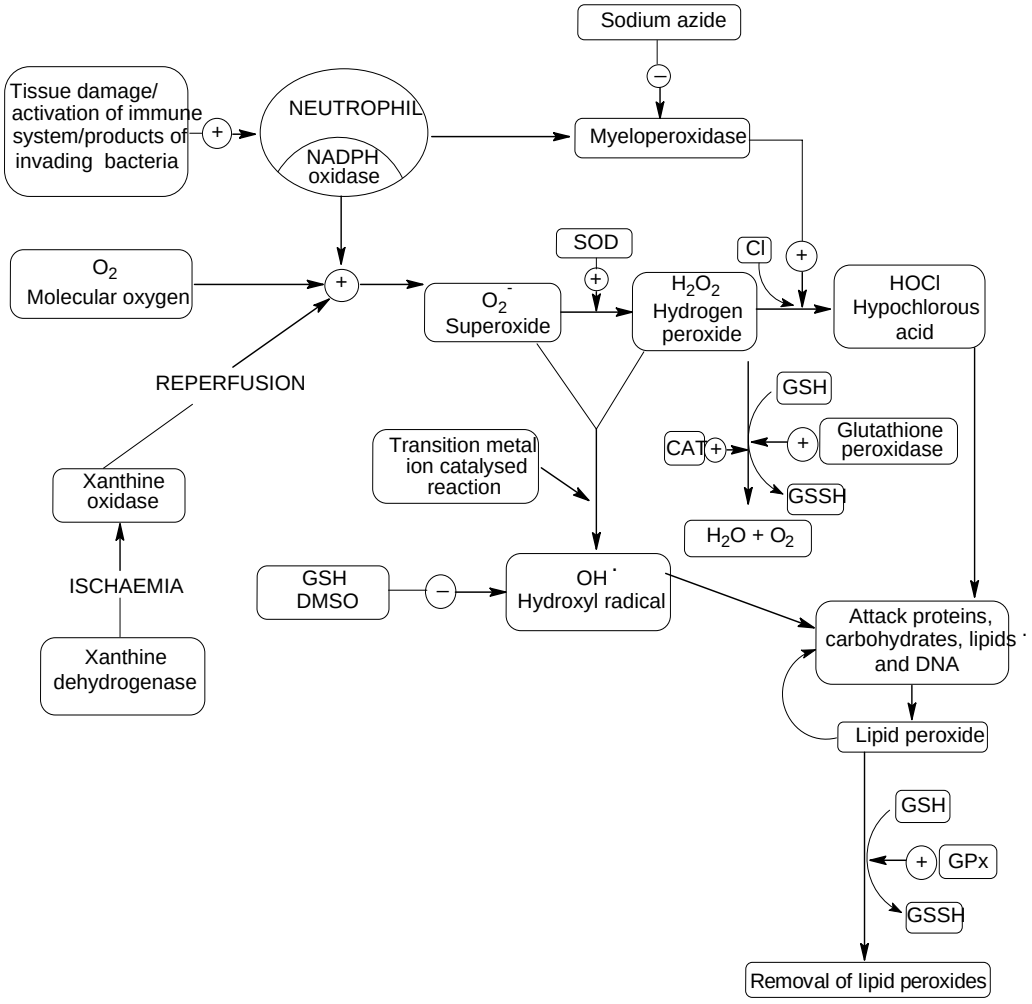
Reduced glutathione (GSH)

Glutathione (GSH) is a tripeptide and a powerful antioxidant present within the cytosol of cells and is the major intracellular nonprotein thiol compound (NPSH). Glutathione is synthesized intracellularly from cysteine, glycine and glutamate. GSH is important in maintaining -SH groups in other molecules including proteins, regulating thiol-disulfide status of the cell, and detoxifying foreign compounds and free radicals. Sulfur containing amino acids like methionine and cysteine are precursors of GSH but also provide-SH groups to react with H_2O_2 and the $OH^{\bullet}$ radical and may prevent tissue damage. GSH is capable of scavenging ROS directly or enzymatically via GPx (Demir *et al.,* 2003; Ali *et al*., 1996).

Vitamin C and E

The number of non-enzymatic endogenous antioxidant mechanisms also exists within normal cells such as vitamins C and E. They can react with free radicals to form radicals themselves which are less reactive than the radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals. It is possible that these vitamins are consumed in the process of lipid peroxidation induced by

oxygen radicals in ischemia reperfusion to prevent the development of tissue damage (Ali *et al*., 1996). Removal of excess reactive species or suppression of their generation by antioxidants may be effective in preventing oxidative cell death (Tian *et al*., 2007). Antioxidants acts as a radical scavengers and inhibit lipid peroxidation and other free radical mediated process and protect the human body from several disease attributed to the reaction of radicals (Repetto and Llesuy, 2002).



+ , Catalyses reaction; – , inhibites enzyme or scavenges reactive oxygen metabolites ; NADPH, nicotinamide adenine dinucleotide phosphate; DMSO, dimethylsulphoxide; SOD, superoxide dimutase; CAT, catalase, GPx, glutathione peroxidase; GSH, reduced glutathione; GSSH, oxidized glutathione.

Fig 4: Pathway of reactive oxygen metabolite formation and site of action of some antioxidants (Davies and Rampton, 1994)

1.2.7 Role of phytomedicine in peptic ulcer

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body (Kamboj, 2000). Natural products from plants are a rich resource used for centuries to cure various ailments. The use of bioactive plant-derived compounds is on the rise, because the main preoccupation with the use of synthetic drugs is the side effects which can be even more dangerous than the diseases they claim to cure. In contrast, plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness and proved to be safe, better patient tolerance, relatively less expensive and globally competitive. So, a retrospect of the healing power of plants and a return to natural remedies is an absolute requirement of our time (Sannomiya *et al*., 2007). Therefore, alternative approach in recent days is the research of medicaments from traditional medicine. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the

offensive factors serving as a tool in the prevention of peptic ulcer (Jainu and Devi, 2006).

1.2.8 Combination therapy

For many years, the therapeutic superiority of a plant drug combination over a mono extract had only the support of practical experiences. The first approach toward rationalizing this multi drug therapeutic concept was made by Berenbaum (1989). He described that 'a total effect of a combination is greater than expected from the sum of the effects of the single' and 'synergy is deemed present if the effect of a combination is greater than that of each of the individual agents'. Now a day's combination therapy is one of the important practices in treatment of various diseases and combination therapy shows greater effect and efficacy than the single drug treatment (Wagner, 2006). Various studies showed that when herbal product is combined with modern drugs it produces better effect and reduces toxic effect resulting in better efficacy and treatment (Muniappan and Sundararaj, 2003).

1.3 DRUG PROFILE

Gallic acid

Gallic acid belongs to the group of polyhydroxy phenolic compound. It is widely distributed in plant kingdom mainly in fruits and vegetables. Gallic acid is present as free molecule or as a part of the tannin molecule.

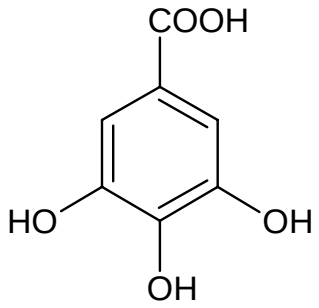
Category

It is belongs to a class of phenolic compound.

Sources

Gallic acid is found in many natural products like gallnuts, grapes, tea leaves, green tea, hops, oak bark, apple-peels, pineapples, bananas, lemons, strawberries, sumac, red and white wine, asparagus, broccoli and aubergine (Madlener *et al.,* 2007).

Molecular structure and chemical name



Gallic acid (3,4,5- trihydroxy benzoic acid ($C_7H_6O_5$))

Uses

Gallic acid is a potent antioxidant and has wide range of biological activities (Yen *et al.,* 2002). It is used as neuroprotective, anti-obesity, hepatoprotective, anti allergic, anti-inflammatory,

antifungal, antimicrobial and antiasthmatic agent (Ban *et al.,* 2008; Hsu and Yen, 2007; Jadon *et al.,* 2007; Kim *et al.,* 2006; Ahn *et al.,* 2005; Dorsch *et al.,* 1992). Gallic acid is important for its anticancer, chemoprotective, antitumor activity (Faried *et al.,* 2007; Veluri *et al.,* 2006; Prasad *et al.,* 2006; Isuzugawa *et al.,* 2001, Inoue *et al.,* 1994). Different plant and fruit extracts contains gallic acid as an important constituent, showing mutagenic, anti-diabetic and anti-ulcer activity (Sannomiya *et al.,* 2007; Govindarajan *et al.,* 2006; Sridhar *et al.,* 2005).

2. LITERATURE REVIEW

Umamaheswari *et al.*, (2007) evaluated the antiulcer activity of the ethanolic extract of *Jasminum grandiflorum* L. leaves (JGLE, 100 and 200 mg/kg, *b.w.,* p.o) on aspirin + pylorus ligation and alcohol induced acute gastric ulcer models and ulcer-healing activity using acetic acid induced chronic ulcer model in rats. The antioxidant activity of JGLE was also studied using DPPH assay, reductive ability, superoxide anion scavenging activity, nitric oxide scavenging activity and total phenolic content of the extract. The ulcerative lesion index significantly decreased ($P<0.01$) in all the three models in rats as compared to the standard drug famotidine (20 mg/kg, *b.w*. orally). A decrease in the gastric fluid and total acidity and an increase in the pH of the gastric fluid in aspirin plus pylorus ligation rats proved the antisecretary activity of JGLE. JGLE completely healed the ulcer within 20 days of treatment in acetic acid induced model like antiulcer activity, the free radical scavenging activities of JGLE depends on concentration and increased with increasing amount of the extract. The result suggests that the leaves of *Jasminum grandiflorum* possess potential antiulcer activity, which may be attributed to its antioxidant mechanism of action.

Hsu and Yen (2007) investigated anti-obesity effect of gallic acid and beneficial effect of gallic acid in suppression of high-fat diet (HFD)-induced dyslipidaemia, hepatosteatosis and oxidative stress in male *Wistar* rats by feeding them a HFD. Gallic acid (50 and

100 mg/kg) was given to two groups as a supplement for a period of 10 weeks. A significant decrease in body weight, organ weight of the liver and adipose tissue weights of peritoneal and epididymal tissues was observed in the HFD + gallic acid groups when compared with HFD group. Serum triacylglycerol (TAG), phospholipid, total cholesterol, LDL-cholesterol, insulin and leptin levels were also decreased in similar manner. Histological study showed that the lipid droplets of rats with HFD + gallic acid diets were significantly smaller than those with HFD diets. The consumption of gallic acid also reduced oxidative stress and GSSG content and enhanced the levels of glutathione, glutathione peroxidase, glutathione reductase and glutathione S-transferase in the hepatic tissue of rats with HFD-induced obesity.

Jadon *et al.*, (2007) reported that *Terminalia belerica* Roxb. and gallic acid protects liver and kidney against carbon tetrachloride induced damage in albino rats. After administration of carbon tetrachloride a significant increase in the serum transaminases, serum alkaline phosphatase activity and hepatic lipid peroxidation and a significant depletion in reduced glutathione level were observed. A minimum elevation was found in protein content and a significant fall was observed in glycogen content of liver and kidney after toxicant exposure. Adenosine triphosphatase and succinic dehydrogenase activities were also inhibited significantly in both the organs after toxicity. Treatment with plant extract (200, 400 and 800 mg/kg, p.o.)

and gallic acid (50, 100 and 200 mg/kg, p.o.) showed dose-dependent recovery in all these biochemical parameters but the effect was more pronounced with gallic acid.

Madlener *et al.*, (2007) examined cytotoxic and biochemical effects of gallic acid on the human HL-60 promyelocytic leukemia cell line. Gallic acid (GA) is a naturally occurring polyhydroxyphenolic compound and an excellent free radical scavenger. Gallic acid inhibits ribonucleotide reductase by causing significant imbalance of deoxynucleosidetriphosphate (dNTP) pool sizes. Gallic acid (80 mM) induced dose-dependent apoptosis (39% cells) in HL-60 cells and gallic acid (60 mM) attenuated progression from G0/G1 to the S phase of the cell cycle. The IC_{50} values of gallic acid were 3.5 and 4.4 nM for the inhibition of cyclooxygenases I and II, respectively. Highly synergistic growth inhibitory effects could be observed when cells were simultaneously treated with GA and trimidox, another inhibitor of ribinucleotide reductase.

Faried *et al.*, (2007) reported the anticancer effect of gallic acid. Gallic acid, a natural antioxidant isolated from fruits of herbal medicinal plant *Phaleria macrocarpa*. The gallic acid showed significant inhibition of cell proliferation in a series of cancer cell lines and induces apoptosis in esophageal cancer cells (TE-2), but did not show any apoptosis in non cancerous cell (CHEK-1). Gallic acid up-regulated the pro-mechanism of apoptosis protein, Bax and induced caspase-cascade activity in cancer cell; Gallic acid also down regulated anti-apoptosis protein like Bcl-2 and Xiap and induced down

regulation of survival Akt/mTOR pathway. In non-cancerous cells, gallic acid delayed expression of pro-apoptosis related proteins.

Gupta *et al.*, (2007) suggested that gallic acid is a potent inhibitor of brush border sucrase and other disaccharidases and thus could potentially interfere with the digestive functions of the intestine. Gallic acid at concentrations of 0.05 to 0.6 mmol/L inhibited sucrase activity by 34% to 86% at a pH 4.8 to 7.2. But at alkaline pH (7.7-8.5), gallic acid stimulated enzyme activity by 20% to 30% in both rat and rabbit intestines. Gallic acid was a fully competitive inhibitor of rat sucrase at pH 5.5 and 6.8. Gallic acid showed optimum enzyme inhibiting activity at a 0.4-mmol/L concentration, which was 82% in the rat, 83% in LACA/L mice, 50% in BALB/c mice, and 28% in rabbit intestine. The depressed the activities of maltase (42%), trehalase (45%), and lactase (13%) were observed in the rat with gallic acid. The gallic acid (0.4 mmol/l) with 4 mmol/l harmaline (-SH group–reacting reagents) showed that the observed inhibition was additive in nature.

Appeldoorn *et al.*, (2007) reported that gallic acid antagonizes p-selectin–mediated platelet–leukocyte interactions. Gallic acid potently inhibited the binding of a peptide antagonist (IC_{50}, 7.2 µmol/l) and biotin-PAA-Le^a-SO3H, an established high-affinity ligand, to P-selectin (IC_{50}, 85 µmol/l). Gallic acid markedly and dose dependently attenuated the rolling of monocytic HL-60 cells over P-selectin–transfected Chinese hamster ovary cells (EC_{50}, 14.5 µmol/l) under dynamic flow condition and increasing the velocity of

P-selectin– dependent rolling of human blood leukocytes over a platelet monolayer. Gallic acid administration to normolipidemic C57/Bl6 and aged atherosclerotic apolipoprotein E–deficient mice impaired the baseline rolling of conjugates between activated platelets and circulating monocytes over femoral vein endothelium, as judged by online video microscopy.

Sener *et al.*, (2007) reported the protective effect of rosiglitazone against burn – induced oxidative injury of remote organs using rats. Severe burn induces the activation of an inflammatory cascade that contributes to the development of subsequent immunosuppression, increased susceptibility to sepsis, as well as generation of reactive oxygen radicals and lipid peroxidation, leading to multiple organ failure. Significant decrease in GSH level, and significant increases in malondialdehyde level, myeloperoxidase activity and collagen content of tissues observed in severe skin scald injury (30% of total body surface area). Similarly, serum aspartate aminotransferase, alanine aminotransferase and blood urea concentration levels, aswell as lactate dehydrogenase, interleukin-1β and tumor necrosis factor-α were elevated in the burn group as compared to the control group. Rosiglitazone treatment reversed all these biochemical indices. Rosiglitazone possesses anti-inflammatory effect that prevents burn-induced damage in remote organs and protects against organ damage.

Govindarajan *et al.*, (2006) reported the antiulcer and

antimicrobial activity of the 50% aqueous alcoholic extract of the *Anogeissus latifolia* (ALE). The effect of ALE (100 and 200 mg/kg/*b.w.*) was studied on aspirin, cold-resistant stress (CRS), pylorus ligated (PL) and ethanol-induced ulcers. The level of antioxidant enzymes like SOD and catalase along with lipid peroxidation was also studied in CRS-induced ulcers. The ALE significantly inhibited the formation of ulcers induced by physical and chemical agents with a maximum of 84.16% curation (200 mg/kg body weight) in CRS-induced ulcers. ALE decreased levels of LPO and SOD and increased the catalase activity in CRS-induced ulcers. Moderate antibacterial activity and antifungal activity was also observed. High performance thin layer chromatography (HPTLC) showed the presence of gallic acid and ellagic acid (0.95%, w/w, 0.25%, w/w, respectively) in the plant.

Bhave *et al.*, (2006) studied the antiulcer effect of amlodipine and its interaction with H_2 blocker and proton pump inhibitor using pylorus ligation in rats. Amlodipine (0.5 mg/kg and 1.0 mg/kg, i.p.), famotidine (4.0 mg/kg, i.p.) and omeprazole (4.0 mg/kg, i.p) produced significant antiulcer effect which were apparent from the decrease in volume of gastric acid secretion and pH, ulcer index and acidity. But the lower dose of famotidine (1.0 mg/kg, i.p.), omeprazole (1.0 mg/kg, i.p.) and amlodipine (0.25 mg/kg, i.p.) did not show any significant effects. Combined administration of amlodipine (0.25 mg/kg, i.p.) plus famotidine (1.0 mg/kg, i.p.) and amlodipine (0.25 mg/kg, i.p.) plus

omeprazole (1.0 mg/kg, i.p.) showed significant antiulcer effect.

Odabasoglu *et al.*, (2006) investigated the gastroprotective effect of usnic acid (UA) isolated from *Usnea longissima* in indomethacin induced gastric ulcers in rats at doses of 25, 50, 100 and 200 mg/kg body weight. Treatment with indomethacin caused a significant decrease in the levels of SOD, GPx and GSH, and an increase in the LPO ($P<0.05$). Administration of all doses of usnic acid reversed the trend producing a significant increase of SOD, GSH and GPx levels and reduction in LPO level in tissue. UA and ranitidine increased the cNOS activity and lowered the iNOS activity as compared with indomethacin-treated group. However, CAT, GR and MPx activities, increased by indomethacin, were found to be lower in the UA and ranitidine-treated groups. The ulcer index also significantly decreased in usnic acid and ranitidine treated group.

Kesiova *et al.*, (2006) studied the potential antioxidant activity of diphenhydramine (H_1 receptor antagonist) and famotidine (H_2 receptor antagonist). Diphenhydramine inhibited the spontaneous, Fe(II)-induced and Fe(II)/ascorbate-induced lipid peroxidation, while famotidine showed a biphasic concentration-dependent effect on spontaneous lipid peroxidation (a stimulation by 1 mM and an inhibition by 5 mM) and increased Fe(II)-induced- and inhibited Fe(II)/ascorbate-induced lipid peroxidation in the rat liver and brain. Famotidine and diphenhydramine did not alter the catalase activity in all tissue preparations, except for its concentration of 5 mM

(a complete inhibition).

Kim *et al.*, (2006) investigated the anti-allergic action of gallic acid. Immunoglobulin E (IgE) or compound 48/80 -induced histamine release from mast cell was attenuated by gallic acid. Modulation of cAMP and intracellular calcium is the main mechanism to inhibit the histamine release. Gallic acid decreased the phorbol 12-myristate 13-acetate plus calcium ionophore A23187-stimulated pro-inflammatory cytokine gene expression and production of TNF-α and IL-6 in human mast cells. Compound 48/80-induced systemic allergic reaction and IgE-mediated local allergic reaction was also inhibited by gallic acid. Gallic acid inhibits mast cell-derived inflammatory allergic reactions by blocking histamine release and pro-inflammatory cytokine expression.

Prasad *et al.*, (2006) reported the chemopreventive efficacy of gallic acid against ferric nitrilotriacetic acid (Fe-NTA) mediated oxidative stress, toxicity and cell proliferative response in *Wistar* rats. The effect of gallic acid against Fe-NTA-induced carcinogen/drug metabolizing phase I and phase II enzymes, anti-oxidative parameters, kidney markers, tumour promotion markers and lipid peroxidation (LPO) in kidney were observed. Significant depletion in the detoxification and antioxidant enzyme armoury with concomitar elevation in renal LPO, serum creatinine, blood urea nitrogen, hydrogen peroxide generation, ornithine decarboxylase activity and [^{3}H]thymidine incorporation into renal DNA was observed with

intraperitonial administration Fe-NTA (9 mg Fe/kg *b.w.*). Pretreatment with gallic acid (10 and 20 mg/kg *b.w.*) resulted in a significant ($P<0.001$) decrease in the levels of the parameters measured.

Shinno *et al.*, (2005) investigated the effect of gallic on the rat liver microsomal glutathione *S*-transferase (MGST1) *in vitro*. When microsomes were incubated with gallic acid marked increase in MGST1 activity was observed which was prevented in the presence of superoxide dismutase or catalase. Decrease in MGST1 activity in presence of gallic acid was observed when incubation was done with sodium arsenite, a sulfenic acid, reducing agent. The incubation of microsomes with gallic acid in the presence of the NADPH generating system (which generates ROS) increased the MGST1 activity and depressed SOD/CAT. A stable radical, 1,1-diphenyl-2-picrylhydrazyl or ferric chloride prevented the increase of MGST1 activity by gallic acid. These results suggest that the gallic acid acts as a pro-oxidant and activates MGST1 through oxidative modification of the enzyme.

Ahn *et al.*, (2005) studied the antifungal activity of methanolic extract of *Galla rhois* and its active constituents. The biologically active constituents like methyl gallate and gallic acid were isolated and characterized by spectroscopic analysis. Gallic acid exhibited good antifungal activity against *Magnaporthe grisea* and *Erysiphe graminis* and methyl gallate showed activity against *M. grisea, Botrytis cinerea* and *Puccinia recondita*. Both of them were ineffective against *Rhizoctonia solani*. Gallic acid moderately or

significantly inhibited the conidial germination (64%) and appressorium formation (7%) in *M. grisea*. Methyl galate and gallic acid acts on cAMP – related signaling pathway regulating appressorium formation in *M. grisea.*

Aly *et al.*, (2005) examined anti-inflammatory and antiulcer activity of licorice. The antiulcer study was done on indomethacin induced ulceration model. The decrease in mean ulcerated area and percentage of ulceration in the glandular area was observed in licorice and famotidine treated animal groups compared to control-indomethacin group. Antiulcer activity of licorice (100 mg/kg) was found similar to that of famotidine (1.14 mg/kg) which used as a standard drug. The combination therapy of famotidine (0.57 mg/kg) and liqorice (100 mg/kg) showed better anti-ulcer activity than either of them alone.

Rukkumani *et al.*, (2004) examined the protective effect of curcumin and its analog and its analog on alcohol and thermally oxidized sunflower oil (PUFA) induced oxidative stress in *Wistar* albino rats. The liver marker enzymes like glutamyl transferase, alkaline phosphatase, lipid peroxidative indices like TBARS, HP and antioxidants like vitamin C, vitamin E, GSH, SOD, CAT, GPx were estimated. The liver marker enzymes and lipid peroxidative indices were investigated significantly in alcohol, PUFA and alcohol + PUFA groups. Administration of curcumin and curcumin analog abrogated this effect. The antioxidant status which decreased in alcohol, PUFA

and alcohol + PUFA groups was effectively modulated by both curcumin and curcumin analog treatment.

Anoop and Jegadeesan (2003) studied the antiulcerogenic property of aqueous ethanolic extracts of the roots of *Hemidesmus indicus* var. indicus in different animal models like modified pylorus ligated (Shay) rat model and aspirin-induced ulcerogenesis in pylorus ligated rats. The parameters analyzed were gastric volume, ulcer score, pH, free and total acidity and sodium and potassium ion output and bio-chemical estimations like total proteins, total hexoses, hexosamine, fucose, sialic acid and pepsin. Ulcer score was calculated for cysteamine-induced duodenal ulcer model. The roots collected during flowering and vegetative periods showed antiulcer activities in all the parameters studied comparable with that of reference and control groups, proving that roots collected during flowering period were found to be more effective.

Malairajan *et al.*, (2007) evaluated anti-ulcer activity of the ethanol extract of *Toona ciliata* Roemer (heart wood) against aspirin plus pylorus ligation induced gastric ulcer (antisecretory), HCl–ethanol induced ulcer (cytoprotective) and water immersion stress induced ulcer in rats. *Toona ciliata* extract (300 mg/kg p.o.) markedly decreased the incidence of ulcers in all the three models. Significant reduction in gastric volume, free acidity, total acidity and ulcer index were observed in the rat models which was treated with ethanol extract of *Toona ciliata*. Gastroprotective activity (52.94%) was also

observed with the plant extract, whereas standard drug sucralfate showed 94.85%. *Toona ciliata* extract showed protection index 43.0% in water immersion stress induced ulcer,whereas standard drug omeprazole showed an protection index of 100%.

Yen *et al.*, (2002) investigated the antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. The oxidation of deoxyribose induced by Fe^{3+}-EDTA-H_2O_2 was accelerated by gallic acid and ascorbic acid at a concentration of 1.65 mM. They showed no chelating ability toward iron (II). Ascorbic acid and gallic acid exhibited 42.1 and 43.9% scavenging effects on DPPH radicals, respectively and exhibited 60% scavenging effects on hydrogen peroxide at a concentration of 4.17 mM. Ascorbic acid (0.82 mM) and gallic acid (0.6 mM), exhibited the maximal DNA damage. When ascorbic acid and gallic acid were mixed with H_2O_2, a slight inhibitory effect on DNA damage was observed. No toxicity was found in ascorbic acid and gallic acid toward human lymphocytes. The pro-oxidant mechanism for both is most likely due to the strong reducing power and weak metal chelating ability.

Isuzugawa *et al.*, (2001) proved gallic acid induced apoptosis in cancer cell at lower IC_{50} value compared with the values for normal cell. The addition of conditioned medium from cultured hepatocytes results inhibition of apoptosis completely, whereas it is not prevented by conditioned media from tumor cells. Gallic acid induced cell death mediated by ROS and intracellular Ca^{2+}, and it was found that gallic

acid generated hydrogen peroxide in culture medium which induced cell death in dRLh-84 cells. Conditioned media from various tumor cell lines did not contain catalase, and the cytoplasm contained only low levels of catalase. These results show that gallic acid-sensitive cells, including various tumor cells have lack of protective machinery against gallic acid.

Yesilada *et al.,* (2000) studied the antiulcerogenic activity of *Spartium junceum* L. (Fabaceae) in different experimental animal models. The active fraction (saponin fraction) was highly effective in preventing ethanol- and pyloric ligation-induced gastric lesions as well as in inhibiting gastric secretion volume, gastric pH and titratable acidity, but did not affect the hexosamine content of the gastric mucosa. Famotidine (20 mg/kg *b.w*, p.o) was used as a reference standard for this study.

Dias *et al.,* (2000) explored antiulcerogenic activity of crude hydroalcoholic extract (70%) (CHE) of *Rosmarinus officinalis* employing different experimental models and the CHE decreased the ulcerative lesion index produced by indomethacin, ethanol, reserpine in rats. No antisecratory activity was observed on pyloric ligation model. The hydroalcoholic extract increased mucosal non protein sulfhydryl group content and its mechanism has no relationship with nitric oxide and prostaglandins.

George *et al.,* (1999) examined the effect of alpha tocopherol

on gastric ulcers induced by pylorus ligation. Pre-treatment with different dose of alpha tocopherol (400 mg/kg and 600 mg/kg) for 2 weeks protected the gastric mucosa. Alpha tocopherol significantly ($P<0.001$) decreases ulcer index, gastric juice volume and increases pH of gastric juice and mucopolysaccharide contents in dose dependent manner. Vitamin E decreased the gastric ulcer and this effect may be mediated through its antioxidant effect.

Singh and Majumdar (1999) performed antiulcer activity of *Ocimum sanctum* L. (Labiatae) against aspirin, indomethacin, histamine, alcohol, reserpine, serotonin and stress induced ulceration in experimental animal models. Ulcer index was found to decrease significantly for *Ocimum sanctum* (fixed oil) in all the animal models in a dose dependent manner. The inhibitions of gastric secretion, total acidity were observed in aspirin plus pylorus ligated rats. This suggests that *Ocimum sanctum* fixed oil can be considered to be a drug of natural origin.

Venkataranganna *et al.*, (1998) investigated the antiulcer activity of UL-409 (a herbal preparation) and its mechanism of action on different experimental models. UL-409 (600 mg/kg, p.o.) significantly reduced the gastric secretion, total acidity, free acidity and increased carbohydrate:protein ratio which is the gastric mucosal defense mechanism in aspirin + pylorus ligated rats. Pretreatment with UL-409 showed inhibition of alcohol induced contraction of isolated

rat fundus preparation which was reversed by indomethacin proving involvement of cyclo-oxygenase system.

Yesilada *et al.,* (1997) evaluated the antiulcer effect of the flower and flower buds of *Cistus laurifolius* L. The aqueous extract showed good antiulcer activity on pylorus ligation, absolute ethanol, indomethacin, indomethacin plus HCl/EtOH induced gastric and cysteamine induced duodenal ulcer model in rats and mice. The parameters like ulcer index, gastric juice secretion and pH, titrable acidity and acid output, peptic activity and hexosamine content determination was performed. The active fraction showed a potent anti-acid activity.

Inoue *et al.,* (1994) found that gallic acid can induce cell death in promyelocytic leukemia HL-60RG cells. Flow cytometric analysis reversed that the apoptosis was not triggered at a specific phase of the cell cycle and 2 h exposure of gallic acid to HL-60RG cells was enough to induce apoptosis. The gallic acid -induced cell death was mediated by ROS such as hydrogen peroxide, superoxide anion in addition to Ca^{2+} ion, calmodulin-dependent enzymes.

Nakayama *et al.,* (1993) investigated the effects of gallic acid and its esters on H_2O_2-induced cytotoxicity, mutagenicity and SOS response in bacterial assay systems, i.e., the Ames test with *Salmonella typhimurium* TA104 and the SOS chromotest with *E. coli* PQ37. Gallic acid esters found to have the protective effects against

H_2O_2-induced cytotoxicity and no effects on the number of revertant colonies and lowered the SOS induction factor raised by H_2O_2 in Ames and SOS chromo test respectively.

Dorsch *et al.*, (1992) investigated the anti-asthmatic effects of *Galphimia glauca*, gallic acid, and related compounds. The platelet-activating factor (PAF) induced bronchial hypersensitivity was markedly reduced. Gallic acid, methyl gallate and quercetin showed significant effects after a single oral dose of 45 mg/kg, tetragalloyl quinic acid after 5 mg/kg. Continuous treatment of the animals with one certain fraction (GG II, 3 days, 3 x 2 mg/kg) containing all active compounds reduced allergen- and PAF-induced bronchial reactions by more than 70%.

Mukhopadhyaya *et al.*, (1987) showed the anti-ulcerogenic effects of banana pulp powder on aspirin induced gastric and cysteamine induced duodenal ulceration in rats. Banana treated (0.5 g/kg twice daily for 3 days) rats showed significant decrease in gastric juice DNA and protein, increase in total carbohydrate and carbohydrate/protein ratio of gastric juice. In aspirin treated rats opposite effect was observed. The increase in [^{3}H] thymidine incorporation into mucosal cell DNA was found in both the cases. The results tend to confirm that plantain banana powder strengthens mucosal resistance and promotes the healing of ulcers.

Bank *et al.*, (1985) studied the synergistic effect of the combination of ranitidine and sucralphate in prevention of duodenal ulcer. Duodenal ulcers were induced in rats with the secretagogues pentagastrin and bethanechol. Subtherapeutic doses of ranitidine (5 mg/kg/6 h) and sucralphate (50 mg/6 h) yielded an ulcer index which was not significantly different from the control (untreated) ulcer index of 4.3. But ulcer index (0.4 and 0.5) decreased significantly at a therapeutic dose of ranitidine (20 mg/kg) and sucralphate (200 mg/animal). When sub therapeutic doses of ranitidine and sucralphate given in combination the ulcer index reduced significantly at 0.7. The study thus showed sub therapeutic doses of ranitidine and sucralphate given in combination had a synergistic effect equal to therapeutic doses of each of these drugs given alone.

Osborne *et al.*, (1981) showed that gallic acid reduced the antiviral activity of human beta interferon. Cell cultures were incubated with mixtures of either GA and beta interferon (IFN-β) or medium and IFN-β. The cells were subsequently challenged with virus. The virus plaque yields were greater in cells incubated with IFN-β and gallic acid than in cells incubated with IFN-β and medium, indicating that IFN-β had lost antiviral activity in former mixture. The effect of gallic on IFN-β could be reversed with 2-mercaptoethanol due to possible sulfhydryl involvement. The removal of gallic acid from IFN-β by dialysis failed to reverse the reduction in antiviral activity. This suggests that reduction of the activity of IFN-β due to

irreversible interaction between IFN-β and gallic acid.

Niebes (1972) determined the enzyme and its degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects. The activity of 3-glycosaminoglycan hydrolases (β-lucuronidase, β-N-acetylglucosaminidase and arylsulfatase) and glycosaminoglycan and glycoprotein components were significantly increased in varicose patient compared to healthy subjects. The various parameters like determination of sialic acid, total hexoses, fucose, hexosamines, sulphate and total non protein polysaccharides were measured to estimate the mucopolysaccharide components.

3. OBJECTIVE AND PLAN OF WORK

Peptic ulcer is a chronic disease that has become a common health problem affecting a large number of people world wide causing morbidity and mortality. The pathophysiology of this gastro-intestinal disorder is viewed as an imbalance between mucosal defensive factors such as bicarbonate, prostaglandin, nitric oxide, peptides, growth factors and injurious factors like acid, pepsin. Reactive oxygen species (ROS) plays an important role in the pathogenesis of various clinical disorders and gastric damage imposed by physical, chemical and psychological factors contribute to gastric ulceration in humans and animals. Different class of drugs are currently used in the treatment of peptic ulcer like H_2 blockers (famotidine, ranitidine), proton pump inhibitors (omeprazole), M_1 blockers (pirenzepine), protective agents of mucosal barrier (sucralfate) and cytoprotective drugs (misoprostol). Although a wide range of drugs are currently employed in the management of ulcer, most of them exhibit side effects like arrhythmias, impotence, gynaecomastia, artralgia, hypergastrinemia and haemopoeitic changes. Alternative approach in recent days is the research of medicaments from ayurvedic or traditional medicinal system. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer.

Gallic acid (3,4,5- trihydroxy benzoic acid) is a naturally

abundant plant phenolic compound found in grapes, green tea, pineapple, bananas, lemon, straw berries etc. and possess excellent antioxidant, anti-obesity, hepatoprotective, anti-inflammatory, antimicrobial, anti allergic, antifungal, antiasthmatic, chemoprotective and anticancer activity.

Drugs or formulations that possess potent anti-oxidant actions can be more effective in healing experimentally induced gastric ulcers. The multiple effects possessed by gallic acid made us to investigate the antiulcer activity. The objective of our study is to evaluate the antiulcer activity of gallic acid alone and in combination with a known antiulcer agent famotidine using aspirin plus pyloric ligation induced gastric ulcer model in rats.

PLAN OF WORK

- To evaluate the antiulcer activity of gallic acid alone and in combination with famotidine using aspirin plus pyloric ligation induced gastric ulcer model in rats.
- To determine the ulcer index, percentage inhibition, gastric volume and pH of gastric juice.
- Estimation of total and free acidity, peptic activity, total protein and individual carbohydrates like hexoses, hexosamine, fucoses concentration in gastric juice.
- To determine the antioxidant property of the drug by estimation of SOD, CAT, GSH, GR, GPx, TBARS, HP, MPx and glucose 6-phosphate dehydrogenase (G6PD) levels in stomach and liver tissue homogenates.

- Histopathological evaluation of the stomach.
- Statistical analysis using ANOVA followed by Dunnett's test.

4. MATERIALS AND METHODS

4.1 DRUGS & CHEMICALS

Aspirin and famotidine were obtained from Shasan Pharmaceuticals. Gallic acid and bovine serum albumin and were purchased from Loba Chemie Pvt Ltd (Mumbai). Calf thymus DNA was purchased from Sigma Aldrich (USA), Orcinol, D(+) glucosamine hydrochloride, cysteine reagent, *O*-dianisidine hydrochloride and reduced glutathione were procured from Himedia Laboratories Pvt Ltd (Mumbai). Thiobarbituric acid, trichloro acetic acid, fucose, oxidized glutathione, epinephrine and 5, 5- dithiobis (2-nitro benzoic acid) were obtained from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai. All other chemicals used in the study were obtained commercially and were of analytical grade.

4.2 EXPERIMENTAL ANIMALS

Wistar albino rats of either sex weighing between 150-250 g were used. They were maintained under standard laboratory conditions at a temperature of 23 ± 2ºC, with 12 h light - dark cycle, and relative humidity (50 ± 10%). The animals were fed with standard food pellets (Hindustan Lever Ltd) and water *ad libitum*. The rats were deprived of food for 18 h with free excess of water before the experimental procedure. All animal procedures have been approved by Ethical Committee in accordance with animal experimentation and care bearing the number 817/04/AC/CPCSEA.

4.3 ANTIULCER ACTIVITY

Aspirin plus pylorus ligation induced ulcer

The rats were divided into 13 groups (n=6). Gallic acid, aspirin and standard antiulcer drug famotidine were prepared in 0.5% carboxy methyl cellulose suspension as vehicle and administered orally once daily at a volume of 10 ml/kg *b.w.* for 7 days using oral gavage needle. Group I was administered with 0.5% CMC and served as untreated control. Group II received aspirin alone (200 mg/kg *b.w.*) and served as ulcer control group, Group III was treated with test drug gallic acid (200 mg/kg, *b.w.*). Group IV received famotidine (20 mg/kg, *b.w.*) and served as standard. Group V – VII received gallic acid 200 mg/kg *b.w.* in combination with 5, 10, 20 mg/kg famotidine respectively. Group VIII - X were administered with gallic acid 100 mg/kg *b.w.* in combination with 5, 10, 20 mg/kg famotidine respectively. Gallic acid 50 mg/kg *b.w.* in combination with 5, 10, 20 mg/kg famotidine was administered to group XI – XIII respectively.

From days 5 to 7, animals in group II - XIII received aspirin at a dose of 200 mg/kg, 2 h after the administration of respective drug treatment. Animals in all groups were fasted for 18 h after the assigned drug treatment and were anesthetised with anesthetic ether. The abdomen was cut opened by a small midline incision below the xiphoid process and pylorus portion of stomach was lifted out and ligated. Precaution was taken to avoid traction to the blood supply. The stomach was sutured and replaced carefully and the abdomen wall closed in two layers with interrupter sutures. The animals were

deprived of water during post operative period. After, 4 h of pylorus ligation the rats were sacrificed and the stomach was removed and examined and contents were collected for biochemical estimation (Umamaheswari *et al.,* 2007, Sairam *et al.,* 2002).

4.4 BIOCHEMICAL PARAMETERS

The stomach was carefully excised keeping oesophagus closed and opened along the greater curvature and luminal contents were removed. The gastric contents were collected in a test tube and centrifuged. The samples were analyzed for gastric juice volume, pH, free and total acidity. Biochemical estimations like total protein, hexoses, hexosamine, fucose, DNA and pepsin were performed.

4.4.1 Measurement of gastric juice volume and pH

Gastric juice was collected from aspirin plus pylorus ligation induced ulcer rats. The gastric juice thus collected was centrifuged at 3000 rpm for 10 min. The volume of supernatant was measured and expressed as ml/100 g body weight. The pH of the supernatant was measured using digital pH meter (George *et al.,* 1999).

4.4.2 Determination of free and total acidity

An aliquot of 1.0 ml of gastric juice was pipetted out into a 50.0 ml conical flask and 2/3 drops of Topfer's reagent was added to it and titrated with 0.01 N NaOH until all traces of the red colour disappeared and the colour of the solution turned yellowish orange. The volume of 0.01 N NaOH noted. The volume corresponds to free acidity. Then 2/3 drops of phenolphthalein reagent was added and titration was continued until a permanent pink color was developed.

The volume of total alkali consumed was noted which corresponds to total acidity. The free acidity and total acidity was determined using the formula and values are expressed as meq/l per 100 g. (Anoop and Jegadeesan, 2003).

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \quad \text{(meq/l per 100 g)}$$

4.4.3 Estimation of total protein

The dissolved protein present was estimated by treating gastric juice with 90% alcohol (1:9). An aliquot of 0.1 ml of alcoholic precipitate of gastric juice was taken and dissolved in 1.0 ml of 0.1 N NaOH. From this 0.05 ml was taken in another test tube and 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 min for color development. Reading was taken against blank prepared with distilled water at 610 nm in UV – visible spectrophotometer. The protein content was calculated from standard curve prepared with bovine serum albumin and expressed in terms of µg/ml of gastric juice (Lowry *et al*., 1951).

4.4.4 Measurement of pepsin

For each determination four tubes, 1 and 2 containing 5.0 ml of substrate (0.5% BSA), 3 and 4 containing 10.0 ml of trichloro acetic acid (TCA) was placed in the water bath at 37ºC. The gastric juice was mixed with an equal volume of HCl (1:1) at pH 2.1, warmed to 37ºC

and added 1.0 ml of mixture to each tubes 1 and 4, incubated for 15 min and at the end, mixed the contents of tube 1 with tube 3 and allowed to stand in the bath for about 4 min. Contents of tube 1 and tube 3 gives test and contents of tube 2 and tube 4 gives blank. Both the contents were filtered after 25-30 min, 2.0 ml of filtrate was pippeted into 10.0 ml of NaOH, mixed by gentle rotation, then 1.0 ml of phenol was added and again mixed by gentle rotation. After 30 min, the intensity of colour was measured at 680 nm in a spectrophotometer. The difference between test and blank gives a measure of peptic activity. As standard, mixed 2.0 ml of freshly prepared phenol solution containing 50 mg/ml with 10 ml of NaOH and 1.0 ml of phenol reagent was added. After 5-10 min, the colour intensity was measured at 680 nm (Debnath *et al.,* 1974).

4.4.5 Estimation of total carbohydrate content

To 1.0 ml of gastric juice, 9.0 ml of 90% ethanol was added (1:9). The mixture was kept for 10 min and the resultant solution was centrifuged and supernatant was discarded. The precipitate thus obtained was dissolved in 0.5 ml of 0.1 N NaOH. To this, 1.8 ml of 6 N HCl was added. The mixture was hydrolyzed in water bath at 100ºC for 2 h. The hydrolysate thus obtained was neutralized with 5 N NaOH using phenolphthalein as indicator and the volume was made up to 4.5 ml with distilled water and used for the estimation of total hexoses, hexosamine and fucose (Goel *et al.,* 1985).

Hexoses

To 0.4 ml of hydrolysate, 8.5 ml of orcinol reagent was added

by keeping test tube in ice-bath. The mixture was then heated in the boiling water bath at 80ºC for 15 min. It was then cooled under tap water and allowed the color to develop in the dark. The intensity of the color was measured after 25 min at 540 nm against the blank by using distilled water instead of hydrolysate. Total hexoses content was determined from the standard curve of D(+) – galactose – mannose (equal quantities of galactose and mannose in 1.0 ml water) and expressed in µg/ml of gastric juice (Neibes, 1972).

Hexosamine

About 0.5 ml of the hydrolysate fraction was taken in a test tube. To this, 0.5 ml of acetyl-acetone reagent was added. The mixture was heated in water bath at 60ºC for 20 min and then cooled under tap water. To this, 1.5 ml of 90% alcohol was added and allowed for 30 min. The color intensity was measured in a UV – visible spectrophotometer at 530 nm against blank prepared by using distilled water instead of hydrolysate. Hexosamine content was determined from the standard curve prepared by using D(+) glucosamine hydrochloride and concentration has been expressed in µg/ml of gastric juice (Anoop and Jegadeesan, 2003).

Fucose

Three test tubes were taken and in one tube 0.4 ml of distilled water was added which served as control (blank). In each of the other two tubes 0.4 ml of hydrolysates were taken. Then 1.8 ml of sulphuric acid: water (6:1) was added in all the test tubes by keeping the test tubes in ice-cold water bath to prevent breakage due to strong

exothermic reaction. The tubes containing the mixture were then heated in boiling water bath for exactly 3 min and cooled. Then, 0.1 ml of cysteine reagent (3% cysteine hydrochloride in water) was added to the blank and to one of the hydrolysate containing tube (unknown) and cysteine reagent was not added to the last tube containing the hydrolysate (unknown blank). It is then allowed for 90 min to complete the reaction. The absorbance was taken in a UV-visible spectrophotometer at 396 and 430 nm setting zero with distilled water. The optical density for the fucose in the hydrolysate was calculated from the differences in the reading obtained at 396 and 430 nm and subtracting the values without cysteine. This was read against standard curve prepared with D(+)-fucose. The fucose content was expressed in terms of µg/ml of gastric juice (Anoop and Jegadeesan, 2003).

$$\text{True optical density} = \frac{(OD_{396} - OD_{430})^{\text{unknown}} - (OD_{396} - OD_{430})^{\text{unknown blank}}}{(OD_{396} - OD_{430})^{\text{water blank}}}$$

4.4.6 Estimation of DNA

An aliquot of 1.0 ml of gastric juice were added to 0.05 ml of 0.2 M EDTA, 0.1 ml of 3% albumin and 0.1 ml of 50% TCA. The samples were kept frozen for 48 h and then thawed at 37ºC and centrifuged at 2000 rpm for 30 min at 4ºC. The precipitate was taken and washed with 5.0 ml of 5% TCA and then centrifuged. To the precipitate was added 2.0 ml of 5% TCA with constant stirring. The resultant solution was taken in glass stoppered tubes and heated for

15 min at 90ºC in a boiling water bath. The solution was again centrifuged and the precipitate was treated with TCA and similarly hydrolysed and centrifuged. The supernatant after the two hydrolyses were pooled together and the volume noted.

Briefly, 2.0 ml of hydrolysate was taken into a glass stoppered tube containing 0.2 ml of 60% (w/v) perchloric acid. To this, 2.0 ml of 2% diphenylamine reagent was added and then the tube was kept in refrigerator (6-10ºC) for 48 h and the absorbance was read at 600 nm using the blank prepared with 2.0 ml of 5% TCA instead of hydrolysate. The concentration of DNA was determined from a standard curve prepared earlier with calf thymus DNA and expressed as μg/ml of gastric juice/100 g body weight of animal (Mukhopadhyaya *et al.,* 1987).

4.4.7 Ulcer index (UI)

The mucosa was flushed with saline and stomach pinned on a frog board. The lesion in glandular portion examined under a 10x magnifying glass and length was measured using a divider and scale and gastric ulcer was scored. Ulcer index of each animal was calculated by adding the following values and their mean values determined (Dias *et al.,* 2000).

(i) Loss of normal morphology - 1 point
(ii) Discoloration of mucosa - 1 point
(iii) Mucosal edema - 1 point
(iv) Haemorrhages - 1 point
(v) Petechial point (until 9) - 2 point

(vi) Petechial point (> 10) - 3 point

(vii) Ulcer upto 1 mm - n × 2 point

(viii) Ulcer > 1 mm - n × 3 point

(ix) Perforated ulcer - n × 4 point

Where, 'n' is the number of ulcer found.

4.4.8 Percentage inhibition

Percentage inhibition was calculated using the following formula (Malairajan *et al*., 2007)

$$\% \text{ Inhibition} = \frac{UI_{\text{ulcer control}} - UI_{\text{treated}}}{UI_{\text{ulcer control}}} \times 100$$

4.5 BIOCHEMICAL INVESTIGATION OF STOMACH AND LIVER TISSUE

4.5.1 Preparation of stomach mucosa homogenate

The stomach was opened and washed with 0.15 M KCl after the microscopic examination. The mucosa was scraped from the glandular part of stomach, suspended in 5.0 ml of cooled 0.15 M KCl-10 mM potassium phosphate buffer (pH 7.4) containing 0.1% Triton X-100 and centrifuged at 1000 × g for 10 min (Kesiova *et al*., 2006).

4.5.2 Preparation of liver homogenate

The liver was perfused with cooled 0.15 M KCl. The 10% homogenate in 0.15 M KCl-10 mM potassium phosphate buffer, pH 7.4 was centrifuged at 3000 rpm for 10 min (Kesiova *et al.,* 2006).

4.5.3 Estimation of superoxide dismutase (SOD)

SOD activity was determined by the inhibition of autocatalyzed adrenochrome formation in the presence of tissue homogenate at

480 nm. The reaction mixture contained 150 µl of homogenate, 1.8 ml of 30 mM carbonate buffer (pH, 10.2), and 0.7 ml of distilled water and 400 µl of epinephrine (45 mM). Auto oxidation of epinephrine to adrenochrome was performed in a control tube without the homogenate. The activity was expressed as units/mg tissue protein (Misra and Fridovich, 1972).

4.5.4 Estimation of catalase (CAT)

The catalysis of H_2O_2 to H_2O in an incubation mixture adjusted to pH 7.0 was recorded at 254 nm. The reaction mixture contained 2.6 ml of 25 mM potassium phosphate buffer pH 7.0 and 0.1 ml of tissue homogenate and was incubated at 37ºC for 15 min and the reaction was started with the addition of 0.1 ml of 10 mM H_2O_2. The time required for the decrease in absorbance from 0.45 to 0.4 representing the linear portion of the curve was used for the calculation of enzymic activity. One unit of catalase activity was defined as the amount of enzymes causing the decomposition of µM H_2O_2/mg protein/min at pH, 7.0 at 25ºC (Aebi, 1984).

4.5.5 Estimation of glutathione reductase (GR)

The reaction mixture contains 2.1 ml of 0.25 mM potassium phosphate buffer pH 7.6, 0.1 ml of 0.001 M NADPH, 0.2 ml of 0.0165 M oxidized glutathione, 0.1 ml of BSA (10 mg/ml). The reaction was started by the addition of 0.2 ml of tissue homogenate with mixing and the decrease in the absorbance at 340 nm was measured for 3 minutes against a blank. Glutathione reductase activity was expressed as µM NADPH oxidized/min/mg protein at 30ºC (Racker, 1955).

4.5.6 Estimation of reduced glutathione (GSH)

The method was based on the reaction of reduced glutathione with dithionitrobenzoic acid (DTNB) to give a compound that absorbs at 412 nm. Briefly after centrifugation, 0.5 ml of supernatant was taken and mixed with 2.0 ml of 0.3 mol/l di-sodium hydrogen phosphate (Na_2HPO_4) solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml, 1% sodium citrate) was added and the absorbance was measured immediately after mixing at 412 nm. Results were expressed in μM GSH/min/mg protein. (Sener *et al.*, 2007).

4.5.7 Estimation of glutathione peroxidase (GPx)

The reduction of GSSG is coupled to the oxidation of NADPH through glutathione reductase. The reaction mixture contained 100 μl tissue homogenate solution and 800 μl 100 mM/l potassium phosphate buffer (pH 7.4), containing 1 mM/l EDTA, 1 mM/l sodium azide, 0.2 mM/l NADPH, 1 U/ml glutathione reductase and 1 mM/l GSH. After 5 min the reaction was started by the addition of 100 μl 2.6 mM hydrogen peroxide and the absorbance change at 340 nm in 3 min was recorded at 37ºC. Various controls were carried out and suitably subtracted. Glutathione peroxidase activity was expressed as μM NADPH oxidized /min /mg protein at 37ºC (Hsu and Yen, 2007).

4.5.8 Estimation of lipid peroxidative indices

Measurement of thiobarbituric acid reactive substances (TBARS)

For TBARS, 0.1 ml of tissue homogenate (Tris-HCl buffer, pH

7.5) was mixed with 2.0 ml of TBA-TCA-HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA mixed in 1:1:1 ratio). The resultant solution was placed in water bath for 15 min, cooled and centrifuged at 1000 rpm for 10 min. The absorbance of clear supernatant was measured against reference blank at 535 nm. The results were expressed as nM/min/mg tissue protein (Rukkumani *et al*., 2004).

Measurement of hydroperoxides (HP)

For HP, about 0.1 ml of tissue homogenate was treated with 0.9 ml of Fox reagent (88 mg butylated hydroxytoluene (BHT), 7.6 mg xylenol orange and 9.8 mg ammonium ion sulphate were added to 90.0 ml of methanol and 10.0 ml 250 mM sulphuric acid) and incubated at 25ºC for 30 min. The color developed was read at 560 nm spectrophotometrically. The values were expressed as nM/min/mg of protein (Rukkumani *et al*., 2004).

4.5.9 Estimation of myeloperoxidase (MPx) activity

A 100.0 µl of the homogenate was added to 1.9 ml of 10 mM/l phosphate buffer (pH 6.0) and 1.0 ml of 1.5 mM/l *O*-dianisidine hydrochloride containing 0.0005% (w/v) hydrogen peroxide. The changes in absorbance at 450 nm of each sample were recorded on a UV–visible spectrophotometer. MPx activities in tissues were expressed as µM /min /mg protein (Odabasoglu *et al.*, 2006).

4.5.10 Estimation of glucose 6-phosphate dehydrogenase (G6PD)

G6PD was assayed by measuring the increase in absorbance

which occurs at 340 nm due to NADP being reduced to NADPH. This reaction takes place when two electrons were transferred from glucose-6- phosphate to NADP catalyzed by the enzyme G6PD in the reaction.

The reaction mixture consists of 2.0 ml of 0.1 M of Tris-HCl buffer (pH 8.2), 0.1 ml of 0.2 mM NADP, 3.0 ml of $MgCl_2$, 0.4 ml of H_2O and 0.1 ml of enzyme solution. The reaction was started by the addition of 0.1 ml of 6 mM glucose - 6 - phosphate and the increase in absorbance was measured at 340 nm. The activity was expressed in terms of μM /min /mg protein (Sudnikovich *et al.,* 1963).

4.6 HISTOPATHOLOGY

Stomachs were immersed in 10% formalin for 24 h for histopathological examination. The central part of the damaged or ulcerated tissue if present was cut in half along the long diameter. In case of whole stomach protected from the ulcer, then any section was taken from basal part. After processing, the tissue was embedded in paraffin and cut into 5 μm thick sections. The sections were stained with haematoxylin-eosin for histopathological examination (Yesilada *et al.,* 1997).

4.7 STATISTICAL ANALYSIS

Values are expressed as mean ± standard error mean (S.E.M) and analyzed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test. $P<0.05$ were considered statistically significant.

5. RESULTS

In the present study antiulcerogenic activity of gallic acid alone and in combination with famotidine was evaluated using aspirin plus pylorus ligation induced ulcer model. Aspirin plus pylorus ligation induced animals showed extensive gastric lesions that were confined to the glandular portion of the stomach which is evidenced by an increase in ulcer index when compared with untreated control ($P<0.01$) (Table 1). The higher dose combinations like GA 200 + FM 10 and GA 200 + FM 20 exhibited a marked reduction in ulcer which is evidenced by reduction in ulcer index and an increase in percentage of inhibition. Combination of GA 100 + FM 10 and GA 100 + FM 20 resulted in a similar decrease in ulcer index and increase of percentage inhibition and the ulcer healing effect was found to be better than the individual agents when administered alone and the result was found to be statistically significant with $P<0.01$. Combinations of GA 200 + FM 5 and GA 50 + FM 20 also produced effects greater than their individual effects. However, lower dose combinations resulted in a decrease of ulcer protection effects when compared to higher dose combinations and was found to be lesser than the individual agents administered alone (Table 1).

Table 1 shows significant increase in gastric juice volume and decrease in gastric pH in ulcer control rats when compared to control which received vehicle alone. Oral treatments with various combinations of gallic acid and famotidine for 7 days lead to significant ($P<0.01$) decrease of gastric juice volume when compared

to individual treatment. Gastric pH was also elevated among the combination treatment and the increase in pH was greater than their individual administration showing the acid neutralization capacity of the test compounds. Almost all the combinations raised the pH above 3.0 and the results were statistically significant ($P<0.01$). However, GA 50 + FM 5 coadministration did not significantly improve the pH (1.58) on comparison with ulcer control which had pH of 1.17.

Significant increase in total and free acidity, pepsin content, DNA content and total protein concentration was observed in ulcer control rats when compared to control. The levels of total and free acidity, pepsin activity and DNA concentration in the gastric juice of animals which received varying dose of GA + FM was found to decrease dramatically proving the efficacy of combination treatment. This decrease was significantly higher ($P<0.01$) than the values obtained upon individual administration of gallic acid and famotidine (Table 2).

The individual carbohydrate contents like total hexoses, hexosamine and fucose concentration was also decreased in ulcer control rats significantly ($P<0.01$) when compared to normal untreated rats. Pretreatment with gallic acid and famotidine for 7 days showed an pronounced decrease in total protein content of gastric juice when compared against ulcer control. Among the combinations GA 200 + FM 10, GA 200 + FM 20, GA 100 + FM 20 and GA 50 + FM 20 exhibited better reduction in protein content (Table 3). All the drug combination significantly ($P<0.01$) increased the

individual carbohydrate like hexoses, hexosamine and fucose indicating the increase in glycoprotein content when compared to ulcer control. However, the combination of GA 50 + FM 5 did not produce any significant increase in hexoses and hexosamine content (Table 3).

Table 4 shows the level of antioxidant enzymes in the stomach homogenates of animals treated with gallic acid, famotidine and different combinations of both agents at varying dose levels. A significant decrease ($P<0.01$) in the levels of enzymatic and non-enzymatic antioxidant enzymes like SOD, CAT, GSH, GPx, GR and G6PD were observed in ulcer control animals on comparison with control untreated animals. Administration of gallic acid, famotidine alone and in suitable combinations tends to attenuate the levels of all antioxidant enzymes justifying the protection offered by them in conditions of gastric damage. The results obtained were found to be statistically significant ($P<0.01$) with higher protection rate as compared to their individual treatments. The levels MDA and HP, the end product of lipid peroxidation parameters were significantly ($P<0.01$) elevated in the ulcerative stomach homogenate (Table 5). A similar increment in level of MPx was also observed in ulcer control. These high levels of MDA, HP and MPx were profoundly reduced ($P<0.01$) among all the high and lower dose combination groups when compared to the animals which received only gallic acid and famotidine.

The antioxidant enzyme activity was also investigated using the liver tissue in order to check the oxidative stress in liver during gastric ulcer. The results obtained by the effect of the drugs on different antioxidant parameters are tabulated in Table 6 and 7. The ulcer control group showed significant ($P<0.01$) depletion in the levels of SOD, CAT, GSH, GR, GPx and G6PD and increase in MDA, HP and MPx, depicting the oxidative stress in liver during gastric ulcer. Pretreatment with gallic acid, famotidine and all the combinations showed significant ($P<0.01$) increase in levels of SOD, CAT, GSH, GR, GPx and G6PD and decrease in MDA, HP and MPx levels proving the protective nature of test compound.

Microscopic observation of control rats showed normal appearance of gastric mucosa (Fig. 7). Ulcer control animals (Fig. 8a) showed ulcer crater with distorted gastric glands and damaged mucosal epithelium, with inflammation and congestion in the wall of ulcerated stomach. Protection against these histopathological changes was observed by apparent epithelializations, glandular organization, maintenance of mucosa and reduced ulcer size in drug treated groups. The histopathological picture of famotidine and gallic acid showed less inflamed tissue with no ulceration (Fig. 8b, 8c). Combinations like GA 200 + FM 10, GA 200 + FM 20, GA 100 + FM 20 also showed no ulceration. The other combinations were also found to be effective with mild inflammation in tissue and no ulceration.

Table 1: Effect of gallic acid and its combinations on gastric parameters in aspirin plus pylorus ligation induced ulcer rats

Group	Ulcer Index	Percentage Inhibition (%)	Volume of gastric juice (ml/100g)	pH of gastric juice
Control	**0.00 ± 00**	**-**	**0.66 ± 0.06**	**2.00 ± 0.06**
Ulcer Control	**32.33 ± 2.33** [b]	**–**	**4.81 ± 0.54** [b]	**1.17 ± 0.17** [b]
GA (200 mg/kg)	**7.81 ± 1.28** [b]	**75.84**	**2.50 ± 0.10** [a]	**2.32 ± 0.16** [b]
FM (20 mg/kg)	**5.83 ± 0.47** [b]	**81.97**	**2.24 ± 0.12** [b]	**2.48 ± 0.07** [b]
GA 200 + FM 5	**5.80 ± 1.07** [b]	**82.03**	**2.33 ± 0.10** [a]	**3.11 ± 0.09** [b]
GA 200 + FM 10	**3.33 ± 0.42** [b]	**89.70**	**1.84 ± 0.07** [b]	**3.23 ± 0.06** [b]
GA 200 + FM 20	**2.33 ± 0.42** [b]	**92.79**	**1.61 ± 0.06** [b]	**3.45 ± 0.09** [b]
GA 100 + FM 5	**8.50 ± 0.76** [b]	**73.71**	**2.40 ± 0.07** [a]	**2.87 ± 0.07** [b]
GA 100 + FM 10	**4.66 ± 0.61** [b]	**85.59**	**2.02 ± 0.09** [b]	**3.11 ± 0.10** [b]
GA 100 + FM 20	**2.66 ± 0.30** [b]	**91.77**	**1.73 ± 0.08** [a]	**3.29 ± 0.11** [b]
GA 50 + FM 5	**13.00 ± 0.89**	**59.78**	**3.15 ±**	**1.58 ± 0.10**

	[a]		**0.17**[a]	[c]
GA 50 + FM 10	**9.72 ± 0.42** [b]	**69.93**	**2.11 ± 0.08** [b]	**2.40 ± 0.07** [b]
GA 50 + FM 20	**5.37 ± 0.47** [b]	**83.39**	**1.18 ± 0.08** [b]	**2.63 ± 0.08** [b]

GA – Gallic acid; FM – Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]P<0.05, [b]P<0.01, c = non significant when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

Table 2: Action of gallic acid and its combinations on total and free acidity, pepsin and DNA concentration of gastric juice

Group	Total acidity (meq/l per 100g)	Free acidity (meq/l per 100g)	Pepsin (µg/ml)	DNA (µg/ml)
Control	**60.33± 1.43**	**38.66 ± 1.93**	**12.42 ± 0.54**	**300.67 ± 20.07**
Ulcer Control	**81.83 ± 1.47**[b]	**52.67 ± 1.76**[b]	**19.08 ± 1.01**[b]	**455.00 ± 12.50**[b]
GA (200 mg/kg)	**61.33 ± 1.12**[b]	**42.67 ± 1.61**[b]	**12.83 ± 0.67**[b]	**325.00 ± 18.90**[b]
FM (20 mg/kg)	**57.80 ± 1.66**[b]	**38.50 ±1.48**[b]	**11.17 ± 0.51**[b]	**280.01 ± 12.20**[b]

GA 200 + FM 5	**60.50 ± 1.83[b]**	**40.66 ± 1.60[b]**	**12.02 ± 0.84[b]**	**299.17 ± 9.78[b]**
GA 200 + FM 10	**53.33 ± 1.12[b]**	**34.66 ± 0.98[b]**	**10.92 ± 0.72[b]**	**272.50 ± 21.90[b]**
GA 200 + FM 20	**49.83 ± 2.21[b]**	**33.00 ± 1.71[b]**	**8.58 ± 0.85[b]**	**229.17 ± 7.68[b]**
GA 100 + FM 5	**61.17 ± 1.08[b]**	**41.83 ± 1.49[a]**	**12.17 ± 0.49[b]**	**317.50 ± 9.90[b]**
GA 100 + FM 10	**56.00 ± 1.88[b]**	**37.00 ± 1.49[b]**	**12.08 ± 0.60[b]**	**269.17 ± 9.26[b]**
GA 100 + FM 20	**51.33 ± 1.10[b]**	**33.67 ± 1.38[b]**	**9.42 ± 0.64[b]**	**231.17 ± 5.94[b]**
GA 50 + FM 5	**68.16 ± 0.98[a]**	**45.66 ± 0.71[a]**	**15.58 ± 0.83[a]**	**355.60 ± 14.60[a]**
GA 50 + FM 10	**58.50 ± 2.28[b]**	**38.17 ± 0.90[b]**	**13.08 ± 0.66[b]**	**289.16 ± 9.44[b]**
GA 50 + FM 20	**54.17 ± 1.89[b]**	**36.33 ± 1.99[b]**	**10.50 ± 0.70[b]**	**251.65 ± 11.00[b]**

GA – Gallic acid; FM – Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]P<0.05, [b]P<0.01, when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

Table 3: Activity of gallic acid and its combinations on protein and mucosal carbohydrate content in gastric juice of aspirin plus pylorus ligated rats

Group	Total Protein (µg/ml)	Hexosamine (µg/ml)	Hexose (µg/ml)	Fucose (µg/ml)
Control	**510.00 ± 17.13**	**374.17 ± 17.84**	**340.00 ± 17.84**	**124.33 ± 2.85**
Ulcer Control	**786.67 ± 25.65[b]**	**220.83 ± 14.28 [b]**	**256.66 ± 13.01[b]**	**88.00 ± 3.0[b]**
GA (200 mg/kg)	**526.66 ± 14.98[b]**	**357.50 ± 14.76[b]**	**328.33 ± 13.45[b]**	**120.11 ± 2.92[b]**
FM (20 mg/kg)	**501.56 ± 9.46[b]**	**365.83 ± 15.41[b]**	**341.66 ± 10.77[b]**	**132.67 ± 4.15[b]**
GA 200 + FM 5	**518.33 ± 16.60[b]**	**350.00 ± 14.14[b]**	**329.16 ± 16.20[b]**	**123.67 ± 3.74[b]**
GA 200 + FM 10	**471.66 ± 14.92[b]**	**395.00 ± 20.25[b]**	**378.33 ± 22.17[b]**	**158.66 ± 6.59[b]**
GA 200 + FM 20	**466.67 ± 17.44[b]**	**399.17 ± 16.30[b]**	**389.16 ± 16.60[b]**	**165.17 ± 6.40 [b]**
GA 100 + FM 5	**576.66 ± 11.74[b]**	**375.00 ± 8.47[b]**	**317.50 ± 4.43[b]**	**116.33 ± 3.48[b]**
GA 100 + FM 10	**490.00 ± 29.10[b]**	**374.17 ± 17.29[b]**	**348.33 ± 11.30[b]**	**140.66 ± 2.95[b]**
GA 100 + FM 20	**478.33 ± 12.49[b]**	**389.17 ± 16.60[b]**	**394.16 ± 10.12[b]**	**143.33 ± 1.91 [b]**
GA 50 + FM 5	**570.80 ± 28.38[a]**	**300.83 ± 13.13[c]**	**301.83 ± 12.21[c]**	**109.30 ± 8.73[a]**
GA 50 + FM 10	**500.00 ± 18.07[b]**	**370.86 ± 13.63[b]**	**298.43 ± 9.87[b]**	**130.00 ± 2.90[b]**
GA 50 + FM 20	**486.66 ± 29.74[b]**	**377.50 ± 14.19[b]**	**360.00 ± 11.62[b]**	**129.66 ± 2.74[b]**

GA – Gallic acid; FM - Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]P<0.05, [b]P<0.01, c = non significant when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

Table 4 : Antioxidant activity of gallic acid and its combination in rat stomach tissue

Group	SOD (μM/min/mg protein)	CAT (μM/min/mg protein)	GPx (μM/min/mg protein)	GR (μM/min/mg protein)	GSH (μM/min/mg protein)	G6PD (μM/min/mg protein)
Control	**1.16 ± 0.02**	**38.13 ± 1.84**	**1.38 ± 0.05**	**0.32 ± 0.01**	**1.47 ± 0.06**	**2.65 ± 0.10**
Ulcer Control	**0.49 ± 0.01[a]**	**31.00 ± 1.07[b]**	**0.92 ± 0.29[b]**	**0.24 ± 0.01 [a]**	**0.93 ± 0.04[a]**	**1.88 ± 0.04[b]**
GA (200 mg/kg)	**1.11 ± 0.14[a]**	**37.65 ± 0.33[b]**	**1.35 ± 0.16[b]**	**0.31 ± 0.04[a]**	**1.40 ± 0.08[b]**	**2.41 ± 0.20[b]**
FM(20 mg/kg)	**1.22 ± 0.07[b]**	**37.23 ± 2.09[b]**	**1.45 ± 0.09[b]**	**0.30 ± 0.02[b]**	**1.51 ± 0.10[a]**	**2.57 ± 0.06[b]**
GA 200 + FM 5	**1.14 ± 0.24[a]**	**37.96 ± 0.27[b]**	**1.40 ± 0.15[a]**	**0.36 ± 0.02[a]**	**1.39 ± 0.07[b]**	**2.50 ± 0.05[b]**
GA 200 + FM 10	**2.12 ± 0.14[b]**	**40.70 ± 2.39[b]**	**1.71 ± 0.04[b]**	**0.39 ± 0.02[b]**	**1.69 ± 0.10[b]**	**2.74 ± 0.07[b]**
GA 200 + FM 20	**2.24 ± 0.18[b]**	**41.31 ±**	**1.76± 0.10[b]**	**0.48 ± 0.04[b]**	**1.98 ± 0.11[b]**	**2.91 ± 0.08[b]**

		2.36[b]				
GA 100 + FM 5	**1.07 ± 0.07[b]**	**37.50 ± 0.16[b]**	**1.38 ± 0.08[a]**	**0.29 ± 0.03[a]**	**1.39 ± 0.06[b]**	**2.47 ± 0.04[b]**
GA 100 + FM 10	**1.78 ± 0.10[b]**	**38.94 ± 0.82[b]**	**1.79 ± 0.14[b]**	**0.36 ± 0.02[b]**	**1.76 ± 0.13[b]**	**2.67 ± 0.06[b]**
GA 100 + FM 20	**2.16 ± 0.18 [b]**	**40.67 ± 0.47[b]**	**1.90 ± 0.11[b]**	**0.46 ± 0.03[b]**	**1.84 ± 0.20[b]**	**2.83 ± 0.08[b]**
GA 50 + FM 5	**1.06 ± 0.05[b]**	**36.30 ± 0.31[a]**	**1.36 ± 0.07[a]**	**0.28 ± 0.01[a]**	**1.21 ± 0.05[b]**	**2.37 ± 0.04[a]**
GA 50 + FM 10	**1.29 ± 0.06[b]**	**38.63 ± 0.34[b]**	**1.46 ± 0.05[b]**	**0.33 ± 0.03[a]**	**1.54 ± 0.16[b]**	**2.61 ± 0.08[b]**
GA 50 + FM 20	**1.69 ± 0.31[b]**	**39.74 ± 0.26[b]**	**1.64 ± 0.13[b]**	**0.36 ± 0.04[b]**	**1.65 ± 0.19[b]**	**2.77 ± 0.08[b]**

GA – Gallic acid; FM - Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]$P<0.05$, [b]$P<0.01$, when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

Table 5: Anti-lipid peroxidative indices and MPx activities in aspirin plus pylorus ligated rats stomach homogenate treated with gallic acid and its combinations

Group	TBARS (nM/min/mg protein)	HP (nM/min/mg protein)	MPx (μM/min/mg protein)
Control	**3.75 ± 0.10**	**6.00 ± 0.23**	**2.31 ± 0.16**
Ulcer Control	**6.07 ± 0.20[b]**	**9.29 ± 0.42[b]**	**3.79 ± 0.18[b]**
GA (200 mg/kg)	**3.81 ± 0.16[b]**	**6.65 ± 0.15[b]**	**2.42 ± 0.11[b]**
FM (20 mg/kg)	**3.57 ± 0.26[b]**	**6.42 ± 0.26[b]**	**2.01 ± 0.15[b]**
GA 200 + FM 5	**3.68 ± 0.22[b]**	**6.68 ± 0.18[b]**	**2.25 ± 0.17[b]**
GA 200 + FM 10	**2.58 ± 0.23[b]**	**4.58 ± 0.27[b]**	**1.63 ± 0.14[b]**
GA 200 + FM 20	**2.96 ± 0.16[b]**	**4.92 ± 0.12[b]**	**1.33 ± 0.15[b]**
GA 100 + FM 5	**3.71 ± 0.19[b]**	**6.80 ± 0.09[a]**	**1.98 ± 0.10[b]**
GA 100 + FM 10	**3.03 ± 0.12[b]**	**5.25 ± 0.14[b]**	**1.76 ± 0.17[b]**
GA 100 + FM 20	**2.61 ± 0.23[b]**	**4.60 ± 0.15[b]**	**1.35 ± 0.14[b]**
GA 50 + FM 5	**4.42 ± 0.10[b]**	**7.26 ± 0.09[a]**	**2.99 ± 0.13[a]**
GA 50 + FM 10	**3.41 ± 0.11[b]**	**6.09 ± 0.17[b]**	**1.95 ± 0.09[b]**
GA 50 + FM 20	**3.12 ± 0.14[b]**	**6.21 ± 0.08[b]**	**1.74 ± 0.11[b]**

GA – Gallic acid; FM - Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]$P<0.05$, [b]$P<0.01$, when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

Table 6: Antioxidant parameters in liver tissue homogenate treated with gallic acid and its combination in pylorus ligated rats

Group	SOD (μM/min/mg protein)	CAT (μM/min/mg protein)	GPx (μM/min/mg protein)	GR (μM/min/mg protein)	GSH (μM/min/mg protein)	G6PD (μM/min/mg protein)
Control	**2.96 ± 0.22**	**81.48 ± 3.45**	**2.71 ±0.17**	**0.64 ± 0.06**	**3.67 ± 0.16**	**4.89 ± 0.26**
Ulcer Control	**1.22 ± 0.07[b]**	**70.23 ± 1.97[a]**	**1.81 ± 0.09[a]**	**0.34 ± 0.03[b]**	**2.03 ± 0.10[b]**	**2.98 ± 0.06[b]**
GA (200 mg/kg)	**2.95 ± 0.14[b]**	**80.52 ± 0.43[a]**	**2.51 ± 0.13[a]**	**0.58 ± 0.06[b]**	**3.58 ± 0.14[a]**	**4.71 ± 0.10[b]**
FM(20 mg/kg)	**3.08 ± 0.17[b]**	**80.97 ± 4.56[b]**	**2.66 ± 0.15[b]**	**0.62 ± 0.03[b]**	**3.76 ± 0.33[b]**	**4.58 ± 0.09[b]**
GA 200 + FM 5	**2.98 ± 0.19[b]**	**80.76 ± 2.20[b]**	**2.61 ± 0.23[b]**	**0.79 ± 0.03[b]**	**3.85 ± 0.21[a]**	**4.99 ± 0.11[b]**
GA 200 + FM 10	**3.44 ± 0.18[b]**	**83.02 ± 2.91[b]**	**3.83 ± 0.11[b]**	**0.90 ± 0.04[b]**	**4.80 ± 0.16[b]**	**5.02 ± 0.09[b]**
GA 200 + FM 20	**3.87 ± 0.14[b]**	**83.32 ± 3.07[b]**	**3.92 ± 0.10[b]**	**1.11± 0.06[b]**	**5.56 ± 0.13[b]**	**5.31 ± 0.16[b]**
GA 100 + FM 5	**2.87 ± 0.15[b]**	**80.56 ± 1.58[b]**	**2.56 ± 0.12[b]**	**0.51 ± 0.03[a]**	**3.50 ± 0.07[a]**	**4.64 ± 0.08[b]**

GA 100 + FM 10	**3.47 ± 0.13[b]**	**82.46 ± 1.36[b]**	**2.99 ± 0.24[b]**	**0.75 ± 0.06[b]**	**4.37 ± 0.21[b]**	**5.06 ± 0.07[b]**
GA 100 + FM 20	**3.50 ± 0.15[b]**	**82.67 ± 0.37[b]**	**3.36 ± 0.12[b]**	**0.98 ± 0.10[b]**	**4.84 ± 0.25[b]**	**5.11 ± 0.07[b]**
GA 50 + FM 5	**1.97 ± 0.18[a]**	**79.80 ± 1.12[a]**	**2.30 ± 0.04[a]**	**0.50 ± 0.03[a]**	**2.98 ± 0.07[a]**	**4.01± 0.13[b]**
GA 50 + FM 10	**3.15 ± 0.09[b]**	**81.19 ± 1.13[b]**	**3.21 ± 0.06[b]**	**0.57 ± 0.07[b]**	**4.11 ± 0.17[b]**	**4.72 ± 0.10[b]**
GA 50 + FM 20	**3.38 ± 0.09[b]**	**82.06 ± 1.16[b]**	**3.80 ± 0.23[b]**	**0.73 ± 0.08[b]**	**4.63 ± 0.11[b]**	**4.86 ± 0.09[b]**

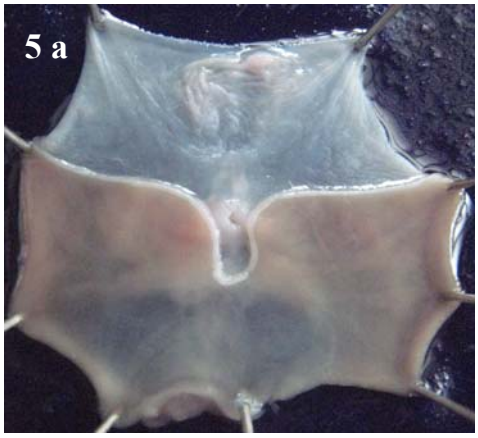
GA – Gallic acid; FM - Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]$P<0.05$, [b]$P<0.01$, when ulcer control was compared with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.

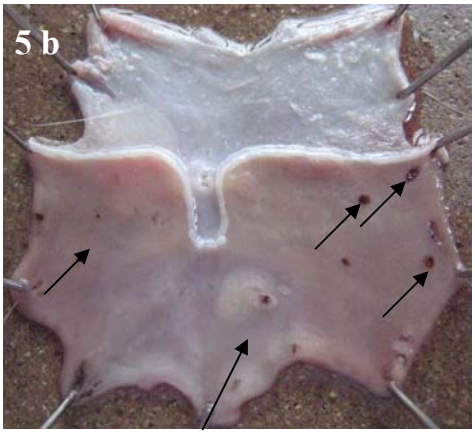
Table 7: Effect of gallic acid alone and in combination on lipid peroxidation indices and MPx activities in aspirin plus pylorus ligated liver tissue.

Group	TBARS (nM/min/mg protein)	HP (nM/min/mg protein)	MPx (μM/min/mg protein)
Control	**3.43 ± 0.79**	**11.14 ± 0.40**	**3.86 ± 0.19**
Ulcer Control	**9.79 ± 0.45[b]**	**15.86 ± 0.62[b]**	**5.96 ± 0.35[b]**
GA (200 mg/kg)	**6.83 ± 0.15[b]**	**11.91 ± 0.18[b]**	**3.67 ± 0.22[b]**
FM (20 mg/kg)	**6.10 ± 0.36[b]**	**11.66 ± 0.63[b]**	**3.26 ± 0.29[b]**
GA 200 + FM 5	**6.73 ± 0.05[b]**	**11.28 ± 0.36[b]**	**3.46 ± 0.19[a]**
GA 200 + FM 10	**4.88 ± 0.34[b]**	**10.17 ± 0.29[b]**	**2.68 ± 0.17[b]**
GA 200 + FM 20	**3.51 ± 0.12[b]**	**9.57 ± 0.21[b]**	**2.44 ± 0.11[b]**
GA 100 + FM 5	**5.13 ± 0.10[b]**	**11.11 ± 0.20[a]**	**3.53 ± 0.13[a]**
GA 100 + FM 10	**5.77 ± 0.07[b]**	**10.54 ± 0.23[b]**	**2.79 ± 0.12[b]**
GA 100 + FM 20	**4.03 ± 0.05[b]**	**10.21 ± 0.19[b]**	**2.51 ± 0.10[b]**
GA 50 + FM 5	**8.34 ± 0.19[b]**	**12.92 ± 0.11[a]**	**4.02 ± 0.07[a]**
GA 50 + FM 10	**6.89 ± 0.31[b]**	**11.43 ± 0.16[b]**	**3.32 ± 0.12[b]**
GA 50 + FM 20	**6.82 ± 0.13[b]**	**10.51 ± 0.14[b]**	**2.93 ± 0.22[b]**

GA – Gallic acid; FM - Famotidine. All values are expressed as mean ± S.E.M.; (n=6) animals in each group. [a]P<0.05, [b]P<0.01, when ulcer control was compared

with control untreated animals and gallic acid, famotidine and combination drug treated group were compared with ulcer control.





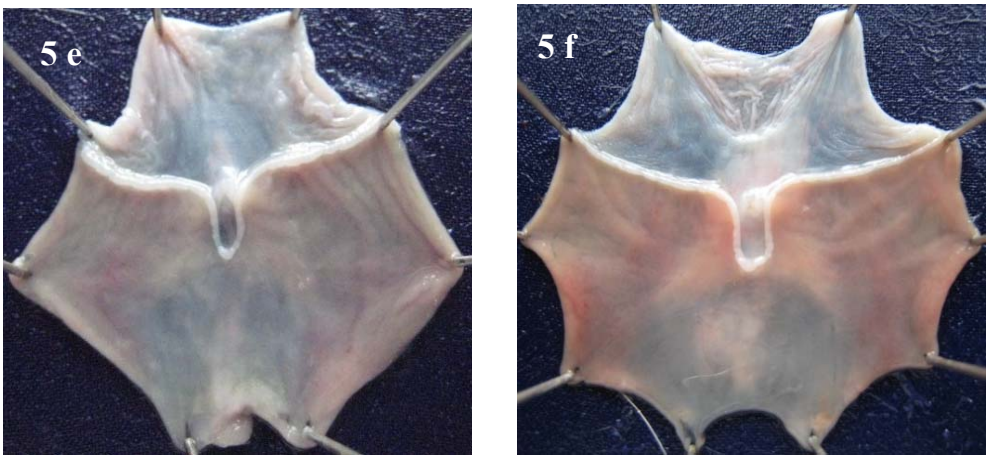


Fig. 5: Stomach of a) untreated control rats ; b) ulcer control rats ; c) famotidine treated rats ; d) gallic acid treated rats; e) GA 200 + FM 5 treated rats ; f) GA 200 + FM 10 treated rats.

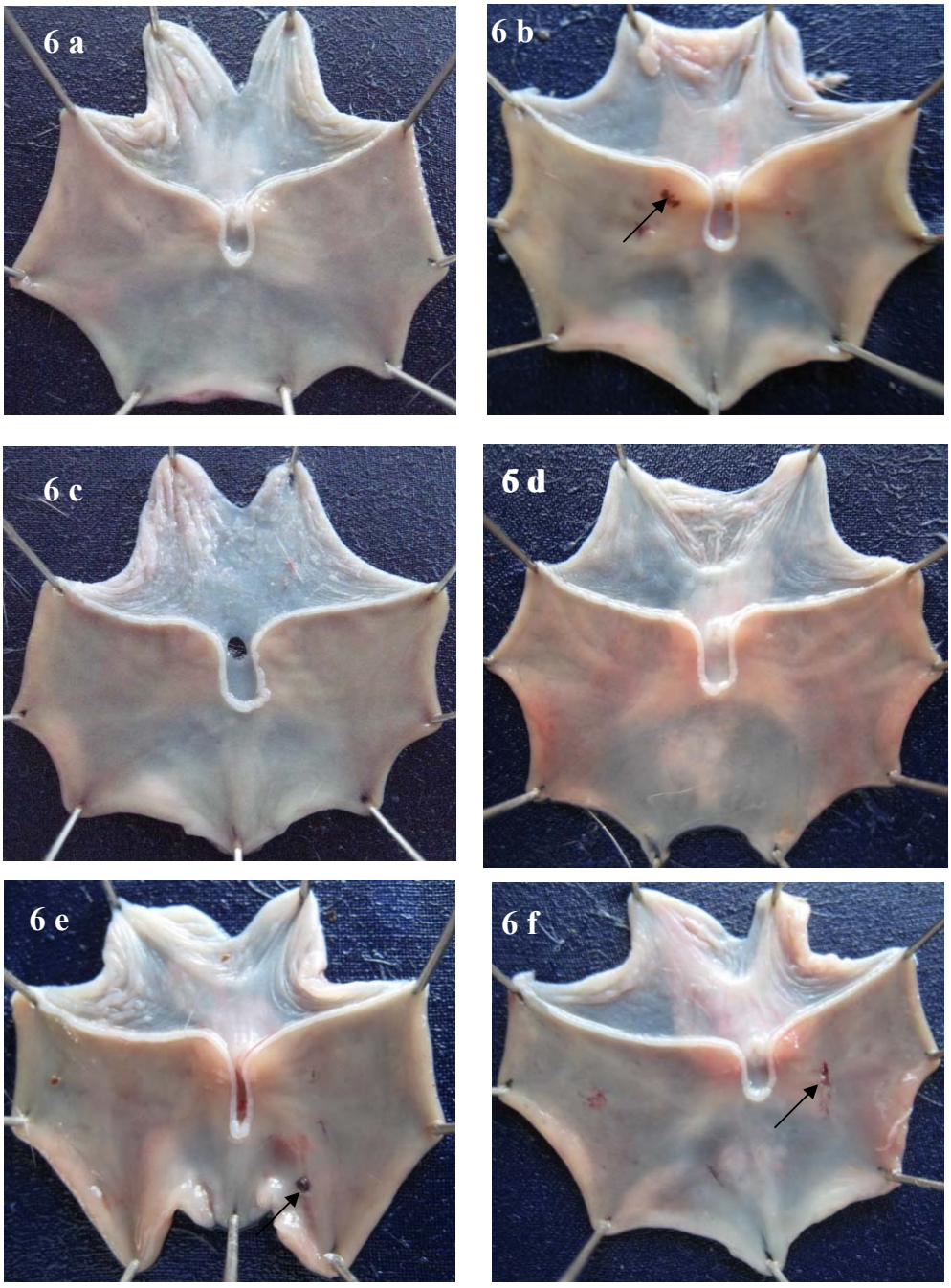


Fig. 6: Stomach of a) GA 200 + FM 20 treated rats ; b) GA 100 + FM 5 treated rats; c) GA 100 + FM 10 treated rats; d) GA 100 + FM 20 treated rats; e) GA 50 + FM 5 treated rats; f) GA 50 + FM 10 treated rats.

Fig. 7: Stomach of a, GA 50 + FM 20 treated rats.

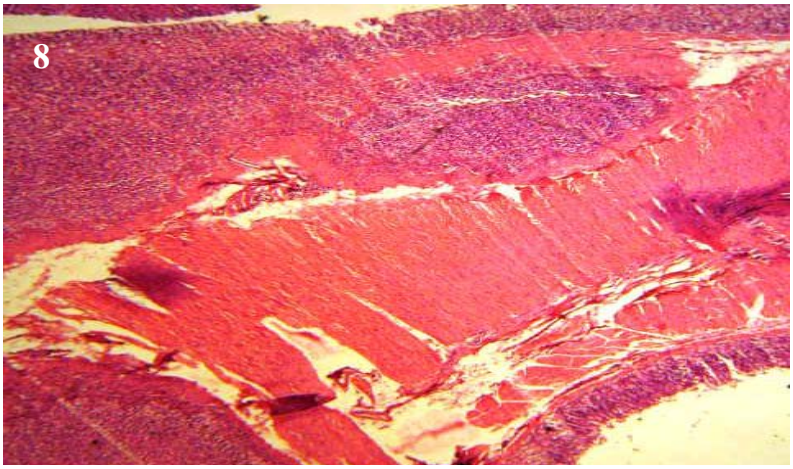


Fig. 8: Histopathological examination of gastric mucosal tissue section of untreated control animals, shows normal gastric mucosa (heamatoxylin and eosin, 100x).

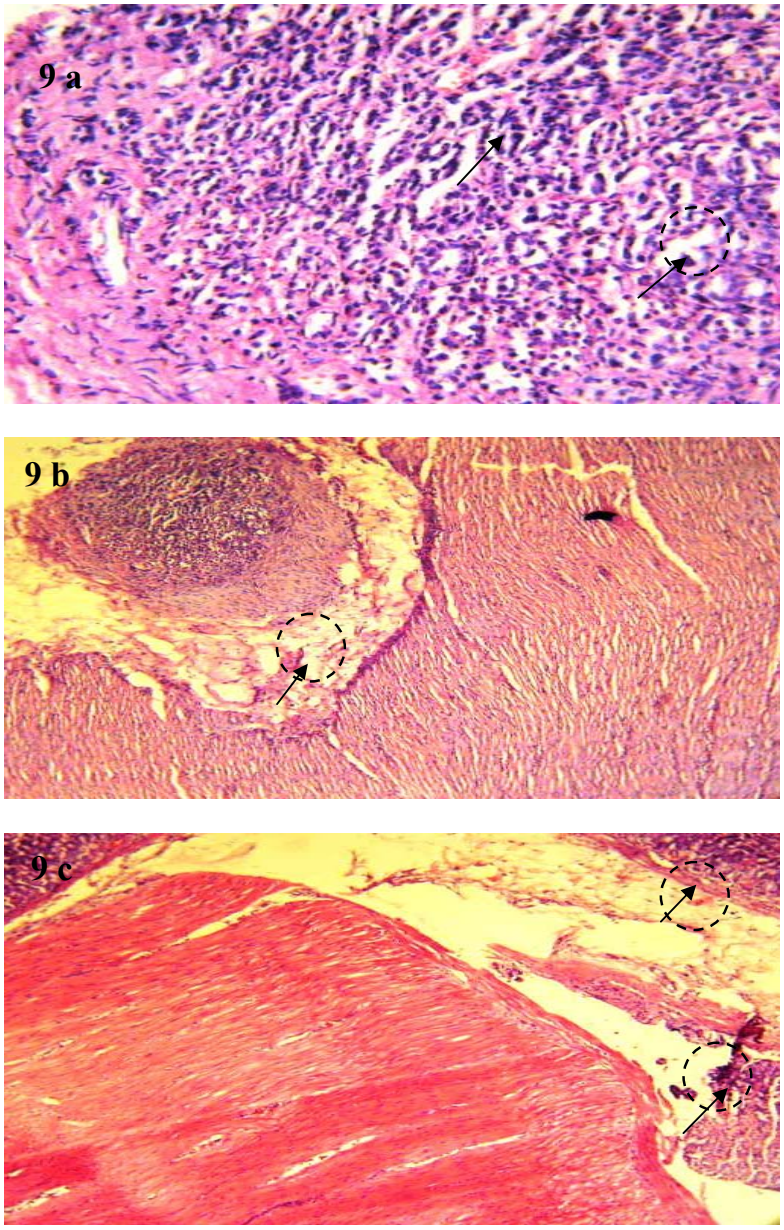


Fig. 9: Histopathological examination of gastric mucosal tissue section of a) ulcerated stomach, shows erosion with distorted gastric glands and damaged mucosal epithelium with inflammation and congestion; b) section of famotidine treated stomach, shows mild inflammation of submucosa; c) section of gallic acid treated stomach shows gastric mucosa with mild inflammation and congestion of submucosa (heamatoxylin and eosin, 100x).

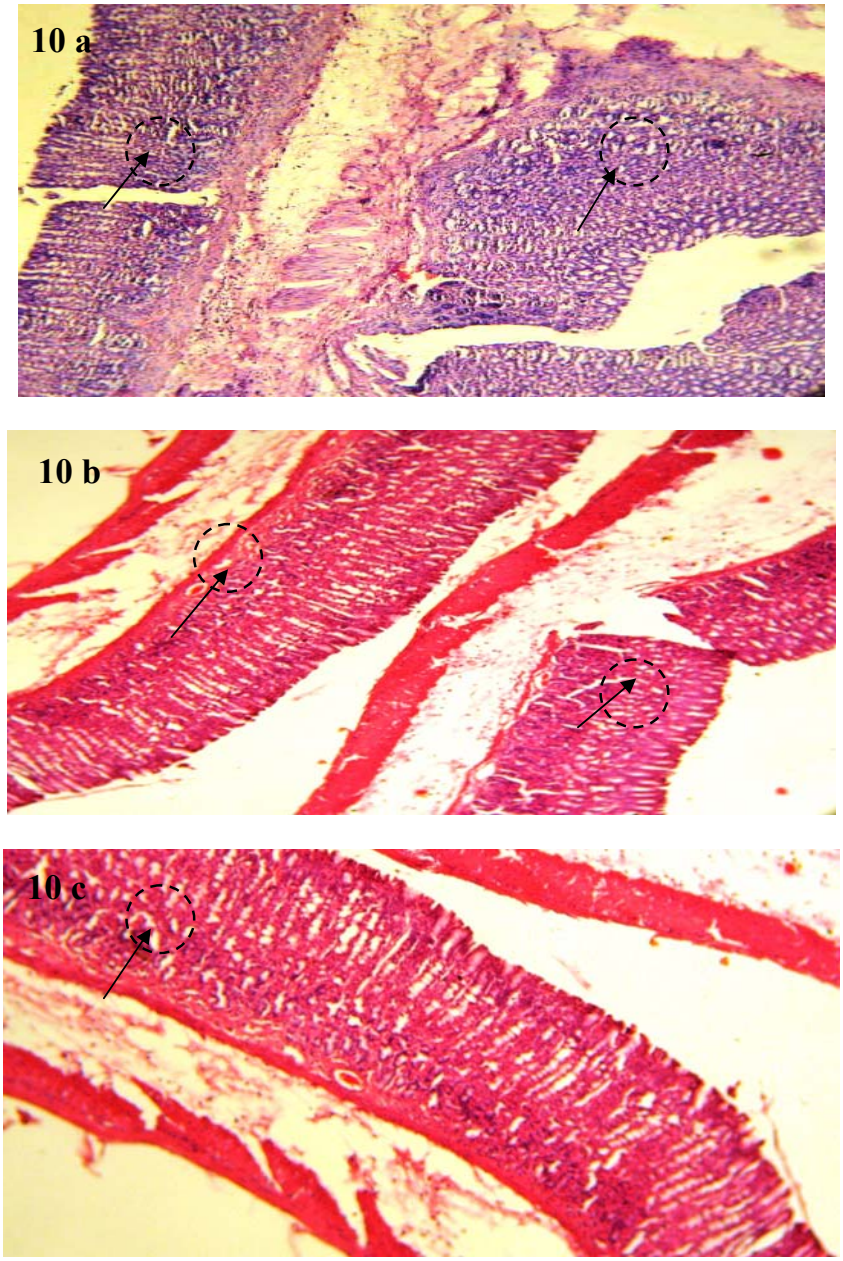


Fig. 10: Histopathological examination of gastric mucosal tissue section of a) GA 200 + FM 5 treated animal stomach, shows mild inflammation of the lamina propria and sumucosa; b) section shows mild inflammation of mucosa, there is no evidence of ulceration, in the gastric mucosa of rats after treatment with GA 200 + FM 10; c) section shows congestion of mucosa,

there is no evidence of ulceration, in the gastric mucosa of rats after treatment with GA 200 + FM 20 (heamatoxylin and eosin, 100x).

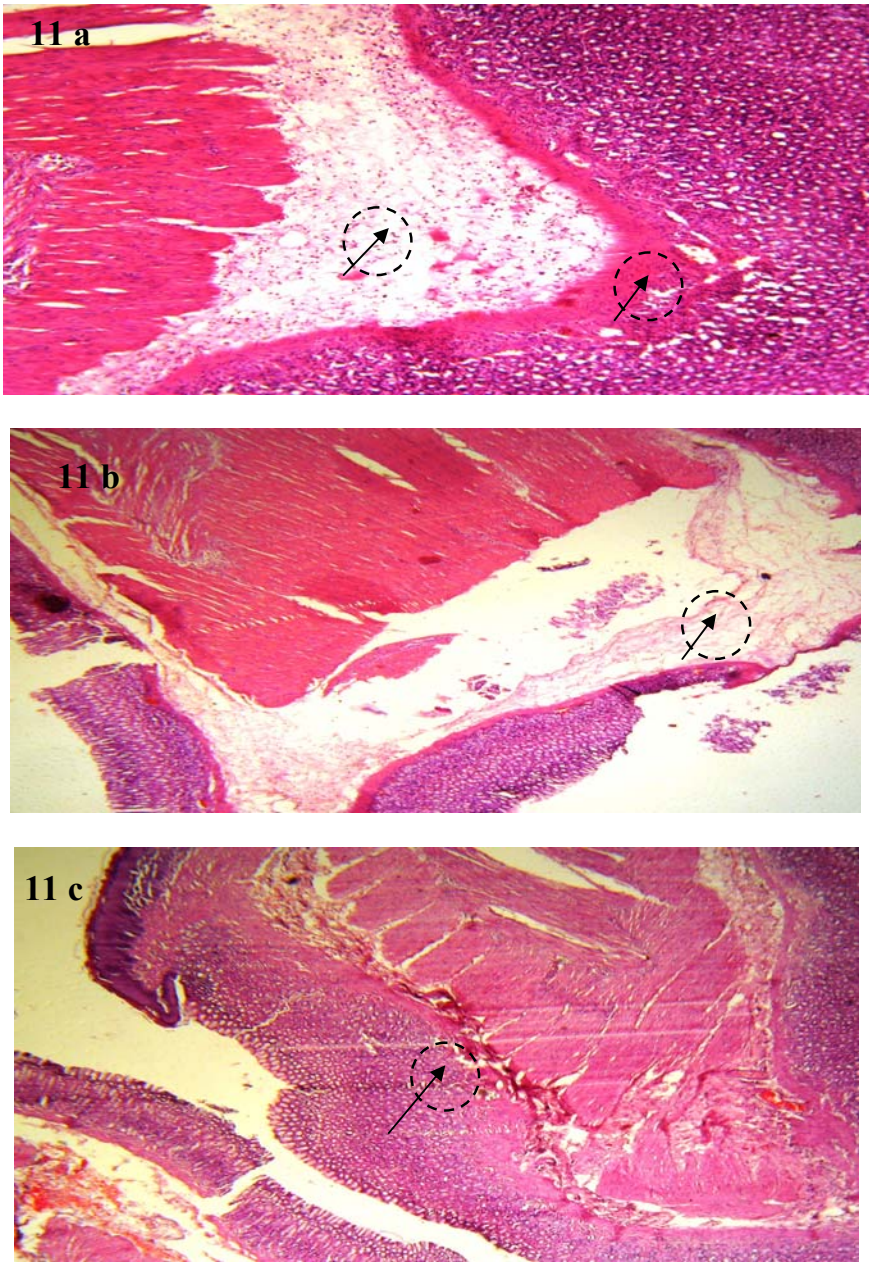


Fig. 11: Histopathological examination of gastric mucosal tissue section of a) section shows gastric tissue with focal inflammation of submucosa after treatment with GA 100 + FM 5; b) section of GA 100 + FM 10 treated stomach, hows inflammation of submucosa no ulcer was found; c) section shows gastroesophagal junction with no ulceration after treatment with GA 100 + FM 20 (heamatoxylin and eosin, 100x).

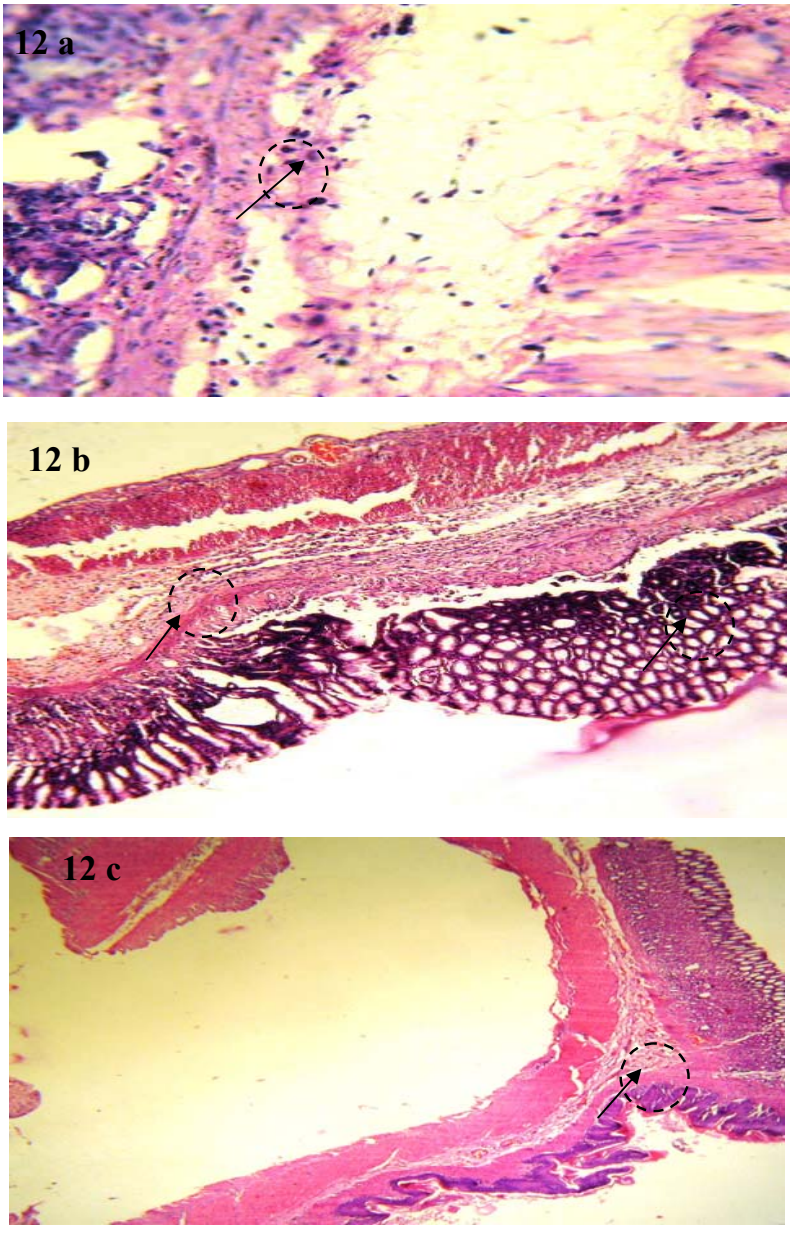


Fig. 12: Histopathological examination of gastric mucosal tissue section of a) section of GA 50+ FM 5 treated stomach, shows inflammation of and congestion of submucosa and lamina propria ; b) section of GA 50+ FM 10 treated stomach, shows gastric mucosa with mild inflammation and edema of submucosa; c) section shows mild inflammation and congestion of gastric mucosa after treatment with GA 50 + FM 20 (heamatoxylin and eosin, 100x).

6. DISCUSSION AND CONCLUSION

In the present study antiulcer effect of the phytoconstituent gallic acid alone and in combination with known antiulcer drug famotidine was investigated in aspirin plus pylorus ligation induced ulcer in rats. NSAIDs like aspirin interfere prostaglandin synthesis through cyclooxygenase pathways and produce neutrophil and oxygen radical dependent microvascular injury leading to mucosal damage (Jafri *et al*., 2001; Maity *et al.,* 1995; Pal and Chaudhuri*,* 1991). Aspirin acts directly by increasing the H^+ ion transport while on the mucosal epithelial cells, it decreases mucin, surface-active phospholipids, bicarbonate secretion (Sairam *et al*., 2003). In pylorus ligated rats accumulation of gastric acid and pepsin secretion are important factors for generation of ulceration. When aspirin was administered to pylorus ligated rats, it further aggravated the acidity and the resistance of the gastric mucosa was decreased thereby imposing extensive damage to the glandular region of the stomach (Sanmugapriya and Venkataraman*.,* 2007; Dharmani *et al.,* 2004). The protective effect of gallic acid as evidenced by percentage inhibition of ulcer against aspirin induced gastric lesions could be due to its cytoprotective effect.

Treatment with gallic acid and its combinations decreased the ulcer index compared to ulcer control group, which is the direct indication of its antiulcer activity. Gallic acid and combination

treatment also decreased volume of acid secretion, peptic activity, total and free acidity and increased pH of the gastric juice compared to ulcer control group proving the antiulcer activity of gallic acid.

Mucus act as defensive factor by preventing physical damage to mucosa leading to lesser degree of ulceration (Sairam *et al*., 2002). The individual carbohydrates such as hexoses, fucose and hexosamine concentrations are the index of mucin content in gastric juice (Annop and Jegadeesan, 2003). Aspirin plus pylorus ligated rat group showed a decrease in the concentration of hexoses, hexosamine and fucose which was reversed by gallic acid and its combinations. Administration of gallic acid resulted in a significant increase in glycoprotein content in gastric mucosa and a net increase in synthesis of mucus contributing to its ulcer protective role.

Increase in protein content of gastric juice resulted in a decrease in total carbohydrate and protein ratio in aspirin plus pylorus ligated rat leading to the damage of gastric mucosa (Jainu *et al.,* 2006). Pretreatment with gallic acid, famotidine and their combinations decreased the plasma protein leakage from gastric mucosa by strengthening the mucosal barrier. The decrease in protein contents of gastric juice by the drugs lead to an increase of total carbohydrate/protein ratio proving the efficacy of combination treatment.

The strengthening of mucosal defense is further exemplified by decrease in cell exfoliation as seen from the reduction in DNA content of the gastric juice (Mukhopadhyaya *et al*., 1987). The reduced level

of DNA in all the drug treated groups further suggests the increase in mucosal defense capacity of the gallic acid.

Free radicals are detrimental to the integrity of biological tissue and mediate their injury (Demir *et al.,* 2003). It is now generally agreed that among various mechanisms, oxidative damage of the gastric mucosal cell membrane by ROS is the major causative factor for gastric ulceration. This decreases the membrane permeability, activities of enzyme and receptors and activation of cells. Drugs that scavenge or inhibit the generation of ROS may be effective in the prevention of gastric damage (Halici *et al*., 2005; Huh *et al*., 2003). Antioxidants constitute the foremost defense system that limit the toxicity associated with free radicals. Gallic acid possess potent antioxidant property (Yen *et al*., 2002), therefore in the present study the antioxidant levels in stomach and liver tissue was investigated to know the protective role of gallic acid in gastric ulcers.

SOD catalyses the reduction of superoxide anion ($O_2^{\bullet -}$) to H_2O_2 and O_2 while CAT converts H_2O_2 to water and molecular oxygen preventing the oxidative damage (Machado-Vieira *et al.,* 2007; Sairam *et al.,* 2003). GPx calalyses the oxidation of GSH to oxidized glutathione (GSSG) at the expense of H_2O_2 and convert it to water. GSSG can be reduced to GSH by GR in presence of NADPH (Liu *et al.,* 2007; Ali *et al*., 1996). The decrease in antioxidant enzymes levels lead to accumulation of ROS by increasing lipid peroxidation. Depletion in SOD, CAT, GR, GPx were observed in the pylorus ligated stomach and liver tissue homogenates. Gallic acid and its

combination with famotidine significantly increased the level of these antioxidant enzymes proving the gastroprotective action by its antioxidant defense mechanism.

GSH present in the stomach plays an important role in maintaining the gastric mucosal cell integrity. GSH depletion is associated with generation of gastric lesion in the rats. GSH is a non enzymatic component and has a central role in antioxidant network (Anandan *et al*., 1999; Altinkaynak *et al*., 2003). In pylorus ligated stomach, decreased GSH level was observed while in drug treated groups an elevated level was noticed substantiating its antioxidant property. A similar increase was also observed in liver tissue homogenate.

Infiltration of neutrophils into gastric mucosal tissue is a critical process in the pathogenesis of gastric ulcer and can be checked by MPx activity. MPx mediates lipid peroxidation in the presence of H_2O_2 and Cl^-. Increase in MPx show an increase in level of neutrophil infiltration into gastric damage tissue (Ohata *et al*., 1999; Odabasoglu *et al*., 2006). An increase in level of MPx was observed in the pylorus ligated stomach tissue and upon administration of gallic acid and its combinations, decreased MPx levels thereby protecting the rats from gastric damage.

Increased lipid peroxidation is one of the major indications of ROS generation in gastric mucosa accompanied by increase of TBARS and HP (Jainu and Devi, 2006; Bafna and Balaraman, 2005). Our data also shows ameliorative increase of lipid peroxides in

pylorus ligated aspirin induced ulcers in rats liver and stomach homogenate. Treatment with gallic acid and its combination resulted in suppression in the levels of TBARS and HP. Polyhydroxy phenolic substances like gallic acid having potent antioxidant properties are anticipated to exert protective effects on ulceration.

G6PD is a cytoplasmic enzyme and acts as a supporter of the primary antioxidant enzymes. It provides coenzymes and substrate to the primary antioxidant enzymes thus playing a protective role against ROS induced oxidative stress. G6PD can slow down the production of cytosolic NADPH by controlling the step from glucose-6-phosphate to 6-phospho-gluconate in pentose phosphate pathway (Ramesh and Pugalendi, 2006; Agarwal and Rami, 2003). G6PD enzyme level significantly decreased ($P<0.05$) in stomach and liver tissue of aspirin plus pylorus ligated rats and were increased by pretreatment with gallic acid and its combinations proving the ulcer protective action of gallic acid.

Antiulcerogenic potential of gallic acid and its combination was further evidenced by the histopathological study of stomach tissue. No ulceration was observed in higher dose combinations like GA 200 + FM 20, GA 200 + FM 10, GA 200 + FM 10 and mild inflammation of submucosa or congesion of submucosa was observed in other treatment groups, suggesting the antiulcer activity of gallic acid and its combinations.

In conclusion, all these result suggest that gallic acid has a gastroprotective effect on gastric lesion induced by aspirin plus

pyrolus ligation. Ulcer healing effect of gallic acid may be due to its increased mucosal defensive and decreased offensive factors. Gallic acid also effectively quenches the free radicals and LPO and positively modulates antioxidant status. The antioxidant property of the test drug might contribute to its antiulcerogenic activity. Combination of both gallic acid and famotidine showed good antiulcer activity, suggesting the interaction of both agents in protection of ulcer.

REFERENCES

Aebi, H. (1984) Catalase *in vitro*. *Methods in Enzymology*, **105**, 121-126.

Agarwal, R. and Rami, A. (2003) Redistribution of glucose-6-phosphate dehydrogenase in response to cerebral ischemia in rat brain. *Indian Journal of Clinical Biochemistry*, **18**, 64-70.

Ahn, Y., Lee, H., Oh, H., Kim, H. and Lee, Y. (2005) Antifungal activity and mode of action of Galla rhois-derived phenolics against phytopathogenic fungi. *Pesticide Biochemistry and Physiology*, **81**, 105-112.

Ali, A.T.M.M., Al-Swayeh, O.A., Al-Rashed, R.S., Al-Mofleh, I.A., Al-Dohayan, A.D. and Al-Tuwaijri, A.S. (1996) Role of oxygen-derived free radicals on gastric mucosal injury induced by ischemia-reperfusion. *The Saudi Journal of Gastroenterology*, **2**, 19-28.

Altinkaynak, K., Suleyman, H. and Akcay, F. (2003) Effect of nimesulide, rofecoxib and celecoxib on gastric tissue glutathione level in rats with indomethacin-induced gastric ulcerations. *Polish Journal of Pharmacology,* **55**, 645-648.

Aly, A.M., Al-Alousi, L. and Salem, H. A. (2005) Licorice: a possible anti-inflammatory and anti-ulcer drug. *AAPS PharmSciTech*, **6**, E74-E82. DOI: 10.1208/pt060113.

Anandan, R., Rekha, R.D., Saravanan, N. and Devaki, T. (1999) Protective effects of *Picrorrhiza kurroa* against HCl/ethanol-induced ulceration in rats. *Fitoterapia*, **70**, 498-501.

Anoop, A. and Jegadeesan, M. (2003) Biochemical studies on the anti-ulcerogenic potential of *Hemidesmus indicus* R.Br. var. *indicus*. *Journal of Ethnopharmacology*, **84**, 149-156.

Appeldoorn, C.C.M., Bonnefoy, A., Lutters, B.C.H., Daenens, K., van Berkel, T.J.C., Hoylaerts, M.F. and Biessen, E.A.L (2005) Gallic acid antagonizes P-selectin-mediated platelet-leukocyte interactions: implications for French paradox. *Circulation*, **111**, 106-112.

Bafna, P.A. and Balaraman, R. (2005) Anti-ulcer and anti-oxidant activity of Pepticare, a herbomineral formulation. *Phytomedicine*, **12**, 264-270.

Balaraman, R., Bafna, P.A. and Kolhapure, S.A. (2004) Antioxidant activity of DHC-1 – a herbal formulation. *Journal of Ethnopharmacology*, **94**, 135-141.

Ban, J.Y., Nguyen, H.T.T., Lee, H., Cho, S.O., Ju, H.S., Kim, J.Y., Bae, K., Song, K. and Seong, Y.H. (2008) Neuroprotective properties of gallic acid from *Sanguisorbae* Radix on amyloid β protein (25-35)-induced toxicity in cultured rat cortical neurons. *Biological & Pharmaceutical Bulletin*, **31**, 149-153.

Bank, S., Zimmerman, H., Smolow, C. and Kranz, V. (1985) Prevention of duodenal ulcers in the rat using a combination of ranitidine and sucralphate in subtherapeutic doses. *Gut*, **26**, 603-606.

Berenguer, B., Sanchez, L.M., Quilez, A., Lopez-Barreiro, M., de Haro, O., Galvez, J. and Martin, M.J. (2006) Protective and antioxidant effects of *Rhizophora mangle* L. against NSAID-induced gastric ulcers. *Journal of Ethnopharmacology*, **103**, 194-200.

Bhave, A.L., Bhatt, J.D. and Hemavathi, K.G. (2006) Antiulcer effect of amlodipine and its interaction with H_2 blocker and proton pump inhibitor in pylorus ligated rats. *Indian Journal of Pharmacology*, **36**, 403-407.

Blandizzi, C., Fornai, M., Colucci, R., Natale, G., Lubrano, V., Vassalle, C., Antonioli, L., Lazzeri, G. and Tacca, M.D. (2005) Lansoprazole prevents experimental gastric injury induced by non-steroidal anti-inflammatoty drugs through a reduction of mucosal oxidative damage. *World Journal of Gastroenterology*, **11**, 4052-4060.

Bowman, W.C. and Rand, M.J. (1980) *Textbook of pharmacology* 2nd ed. London: Oxford Blackwell Scientific Publications. pp. 25.16-25.17.

Brenner, M.G. and Stevens, C.W. (2006) *Pharmacology* 2nd ed, New Delhi: Elsevier. pp. 310-314.

Burks T.F. (1995) Therapy of acid peptic disease. *In*: Munson, P.L. (ed.) *Principles of pharmacology.* New York: Chapman & Hall. pp. 1063-1082.

Chan, F.K.L. and Leung, W.K. (2002) Peptic-ulcer disease. *The Lancet*, **360**, 933-941.

Chatterjee, C.C. (1985) *Human physiology vol I* 11th ed. Calcutta: Medical Allied Agency. pp. 435-438.

Clark, W.G., Brater, D.C. and Johnson, A.R. (1992) Goth's Medical pharmacology 13th ed. St Louis: Mosby Year Book. pp. 507-513.

Davies, G.R. and Rampton, D.S. (1994) *Helicobacter pylori*, free radicals and gastroduodenal disease. *European Journal of Gastroenterology & Hepatology*, **6**, 1-10.

Debnath, P.K., Gode, K.D., Das, G. and Sanyal, A.K. (1974) Effects of propanolol on gastric secretion in albino rats. *British Journal of Pharmacology*, **51**, 213-216.

Demir, S., Yilmaz, M., Koseoglu, M., Akalin, N., Aslan, D. and Aydin, A. (2003) Role of free radicals in peptic ulcer and gastritis. *The Turkish Journal of Gastroenterology*, **14**, 39-43.

Dharmani, P., Kuchibhotla, V.K., Maurya, R., Srivastava, S., Sharma, S. and Patil, G. (2004) Evaluation of anti-ulcerogenic and ulcer-healing properties of *Ocimum sanctum* Linn. *Journal of Ethnopharmacology*, **93**, 197-206.

Dias, P.C., Foglio, M.A, Possenti, A. and de Carvalho, J.E. (2000) Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L. *Journal of Ethnopharmacology,* **69**, 57-62.

Dorsch, W., Bittinger, M., Kaas, A., Muller, A., Kreher, B. and Wagner, H. (1992) Antiasthmatic effects of *Galphimia glauca*, gallic acid, and related compounds prevent allergen- and platelet-activating factor-induced bronchial obstruction as well as bronchial hyperreactivity in guinea pigs. *International Archives of Allergy & Immunology*. **97**, 1-7.

Duggan, J.M. and Duggan, A.E. (2006) The possible causes of the pandemic of peptic ulcer in the late 19th and early 20th century. *The Medical Journal of Australia*, **185**, 667-669.

Faried, A., Kurnia, D., Faried, L.S., Usman, N., Miyazaki, T., Kato, H. and Kuwano, H. (2007) Anticancer effects of gallic acid isolated from Indonesian herbal medicine, *Phaleria macrocarpa* (Scheff.) Boerl, on human cancer cell lines. *International Journal of Oncology*, **30**, 605-613.

George, S., Sathiamoorthy, A. and Sathiamoorthy, S.S. (1999) Effect of alpha tocopherol on gastric ulcers induced by pylorus ligation in rats. *Indian*

Journal of Pharmacology, **31**, 431-433.

Ghosh, B.D. (2007) *Human anatomy for students*. New Delhi: Jaypee Brothers Medical Publishers. pp. 53-59.

Goel, R.K. and Sairam, K. (2002) Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamrabhasma, Asparagus racemosus* and *Zingiber officinale*. *Indian Journal of Pharmacology*, **34**, 100-110.

Goel, R.K., Chakrabarty, A. and Sanyal, A.K. (1985) The effect of biological variables on the antiulcerogenic effect of vegetable plantain banana. *Planta Medica*, **2**, 85-88.

Gooz, M., Gooz, P. and Smolka A.J. (2001) Epithelial and bacterial malalloproteinases and their inhibitors in *H. pylori* infection of human gastric cells. *American Journal of Physiology – Gastrointestinal and Liver Physiology*, **281**, G823-G832.

Govindarajan, R., Vijayakumar, M., Singh, M. and Rao, C.V., Shirwaikar, A., Rawat, A.K.S. and Pushpangadan, P. (2006) Antiulcer and antimicrobial activity of *Anogeissus latifolia*. *Journal of Ethnopharmacology*, **106**, 57-61.

Gupta, N., Gupta, S. and Mahmood, A. (2007) Gallic acid inhibits brush border disaccharidases in mammalian intestine. *Nutrition Research*, **27**, 230-235.

Gurbuz, I. and Yesilada, E. (2007) Evaluation of the anti-ulcerogenic effect of sesquiterpene lactones from *Centaurea solstitialis* L. ssp. *solstitialis* by using various *in vivo* and biochemical techniques. *Journal of Ethnopharmacology*, **112**, 284-291.

Halici, M., Odabasoglu, F., Suleyman, H., Cakir, A., Aslan, A. and

Bayir, Y. (2005) Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. *Phytomedicine*, **17**, 656-662.

Halliwell, B. and Gutteridge, J.M.C. (1999) *Free radicals in biology and medicine* 2nd ed, Oxford: Clarendon Press, pp. 21-35, 148-166.

Harris, E.D. (1992) Regulation of antioxidant enzymes. *The Journal of Nutrition*, **122**, 625-626.

Hiruma-Lima, C.A., Calvo, T.R., Rodrigues, C.M., Andrade, F.D.P., Vilegas, W. and Brito, A.R.M.S. (2006) Antiulcerogenic activity of *Alchornea castaneaefolia*: Effects on somatostatin, gastrin and prostaglandin. *Journal of Ethnopharmacology*, **104**, 215-224.

Hoogerwerf, W.A. and Pasricha, P.J. (2006) Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease. *In*: Brunton, L.L., Lazo, J.S. and Parker, K.L. (eds.) *Goodman & Gilman's The pharmacological basis of therapeutics* 11th ed. NewYork: McGraw-Hill Medical Publishing Division. pp. 1005-1020.

Hsu, C. and Yen, G. (2007) Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats. *British Journal of Nutrition*, **98**, 727-735.

Huh, K., Kwon, T.H., Shin, U.S., Kim, W.B., Ahn, B.O., Oh, T.Y. and Kim, J. (2003) Inhibitory effects of DA-9601 on ethanol-induced gastrohemorrhagic lesions and gastric xanthine oxidase activity in rats. *Journal of Ethnopharmacology*, **88**, 269-273.

Inoue, M., Suzuki, R., Koide, T., Sakaguchi, N., Ogihara, Y. and Yabu, Y. (1994) Antioxidant, gallic acid, induces apoptosis in HL-60RG cells. *Biochemical*

and Biophysical Research Communications, **204**, 898-904.

Isuzugawa, K., Inoue, M. and Ogihara, Y. (2001) Catalase contents in cells determine sensitivity to the apoptosis inducer gallic acid. *Biological & Pharmaceutical Bulletin*, **24**, 1022-1026.

Jadon, A., Bhadauria, M. and Shukla, S. (2007) Protective effect of *Terminalia belerica* Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. *Journal of Ethnopharmacology*, **109**, 214–218.

Jafri, M.A., Farah, Javed, K. and Singh, S. (2001) Evaluation of the gastric antiulcerogenic effect of large cardamom (fruits of *Amomum subulatum* Roxb). *Journal of Ethnopharmacology*, **75**, 89-94.

Jainu, M., and Devi C.S.S. (2004) Effect of Ambrex (an amber based formulation) on gastric mucosal damage: role of antioxidant enzymes and lipid profile. *Indian Journal of Physiology and Pharmacology*, **48**, 343-347.

Jainu, M., and Devi C.S.S. (2006) Antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. *Journal of Ethnopharmacology*, **104**, 156-163.

Jainu, M., Mohan, K.V. and Devi, C.S.S. (2006) Gastroprotective effects of *Cissus quadrangularis* extract in rats with experimentally induced ulcer. *Indian Journal of Medical Research*, **123**, 799-806.

Kamboj, V.P., (2000) Herbal medicine. *Current Science*, **78**, 35-39.

Kesiova, M., Alexandrova, A., Yordanova, N., Kirkova, M. and Todorov, S. (2006) Effects of diphenhydramine and famotidine on

lipid peroxidation and activities of antioxidant enzymes in different rat tissues. *Pharmacological Reports*, **58**, 221-228.

Kim, S., Jun, C., Suk. K., Choi, B., Lim, H., Park, S., Lee, S.H., Shin, H., Kim, D. and Shin, T. (2006) Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. *Toxicological Sciences*, **91**, 123-131.

Liu, C., Chen, N. and Zhang, J. (2007) Protection of PC12 cells from hydrogen peroxide-induced cytotoxicity by salvianolic acid B, a new compound isolated from *Radix Salviae miltiorrhizae*. *Phytomedicine*, **14**, 492-497.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with Folin phenol reagent. *The Journal of Biological Chemistry*, **193**, 265-275.

Lullmann, H., Mohr, K., Ziegler, A. and Bieger, D. (2000) *Color atlas of pharmacology* 2nd ed. New York: Thieme. pp. 166-170.

Machado-Vieira, R., Andreazza, A.C., Viale, C.I., Zanatto, V., Cereser Jr., V., Vargas, R.S., Kapczinski, F., Portela, L.V., Souza, D.O., Salvador, M. and Gentil, V. (2007) Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. *Neuroscience Letters*, **421**, 33-36.

Madlener, S., Illmer, C., Horvath, Z., Saiko, P., Losert, A., Herbacek, I., Grusch, M., Elford, H.L., Krupitza, G., Bernhaus, A., Fritzer-Szekeres, M. And Szekeres, T. (2007) Gallic acid inhibits ribonucleotide reductase and cyclooxygenases in human HL-60 promyelocytic leukemia cells. *Cancer Letters*, **245**, 156–162.

Maity, S., Vedasiromani, J.R. and Ganguly, D.K. (1995) Anti-ulcer effect of the hot water extract of black tea (*Camellia sinensis*).

Journal of Ethnopharmacology, **46**, 167-174.

Malairajan, P., Gopalakrishnan, G., Narasimhan, S., Veni, K.J.K. and Kavimani, S. (2007) Anti-ulcer activity of crude alcoholic extract of *Toona aradis* Roemer (heart wood). *Journal of Ethnopharmacology,* **110**, 348-351.

Misra, H.P. and Fridovich, I. (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*, **247**, 3170-3175.

Mukhopadhyaya, K., Bhattacharya, D., Chakraborty, A., Goel, R.K. and Sanyal, A.K. (1987) Effect of Banana powder (*Musa sapientum* var. *aradisiacal*) on gastric mucosal shedding. *Journal of Ethnopharmacology,* **21**, 11-19.

Muniappan, M. and Sundararaj, T. (2003) Antiinflammatory and antiulcer activities of *Bambusa arundinacea*. *Journal of Ethnopharmacology*, **88**, 161-167.

Nakayama, T., Hiramitsu, M., Osawa, T. and Kawakishi, S. (1993) The protective role of gallic acid esters in bacterial cytotoxicity and SOS responses induced by hydrogen peroxide. *Mutation Research Letters*, **303**, 29-34.

Nelson, D.L. and Cox, M.M. (2005) *Lehninger principles of biochemistry* 4th ed. New York: WH Freeman and Company. pp. 263-264.

Niebes, P. (1972) Determination of enzymes and degradation products of glycosaminoglycan metabolism in the serum of healthy and varicose subjects. *Clinica Chimica Acta,* **42**, 399-408.

Odabasoglu, F., Cakir, A., Suleyman, H., Aslan, A., Bayir, Y., Halici, M. and Kazaz, C. (2006) Gastroprotective and antioxidant effects

of usnic acid on indomethacin-induced gastric ulcer in rats. *Journal of Ethnopharmacology*, **103**, 59-65.

Ohta, Y., Kobayashi, T., Nishida, K., Sasaki, E. and Ishiguro, I. (1999) Preventive effect of Oren-gedoku-to (Huanglian-Jie-Du-Tang) extract on the development of stress-induced acute gastric mucosal lesions in rats. *Journal of Ethnopharmacology,* **67**, 377-384.

Osborne, L.C., Peeler, J.T. and Archer, D.L. (1981) Reduction in antiviral activity of human beta interferon by gallic acid. *Infection and Immunity*, **33**, 769-774.

Pal, S. and Chaudhuri, A.K.N. (1991) Studies on the anti-ulcer activity of a *Bryophyllum pinnatum* leaf extract in experimental animals. *Journal of Ethnopharmacology*, **33**, pp. 97-102.

Parmar, N.S. and Desai, J.K. (1993) A review of the current methodology for the evaluation of gastric and duodenal anti-ulcer agents. *Indian Journal of Pharmacology*, **25**, 120-135.

Prasad, L., Khan, T.H., Jahangir, T. and Sultana, S. (2006) Effect of gallic acid on renal biochemical alterations in male Wistar rats induced by ferric nitriloacetic acid. *Human & Experimental Toxicology*, **25**, 523-529.

Racker, E. (1955) Glutathione reductase from bakers' yeast and beef liver. *The Journal of Biological Chemistry*. **217**, 855-866.

Ramesh, B. and Pugalendi, K.V. (2006) Antihyperglycemic effect of umbelliferone in steptozotocin-diabetic rats. *Journal of Medicinal Food*, **9**,

562-566.

Rang, H.P., Dale, M.M., Ritter, J.M. and Moore, P.K. (2003) *Pharmacology* 5th ed. Edinburgh: Churchill Livingstone. Pp. 367-371.

Rao, C.V., Ojha, S.K., Radhakrishnan, K., Govindarajan, R., Rastogi, S., Mehrotra, S. and Pushpangadan, P. (2004) Antiulcer activity of *Utleria salicifolia* rhizome extract. *Journal of Ethnopharmacology*, **91**, 243-249.

Repetto, M.G. and Llesuy, S.F. (2002) Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian Journal of Medical and Biological Research*, **35**, 523-534.

Rukkumani, R., Aruna, K., Varma, P.S., Rajasekaran, K.N. and Menon, V.P. (2004) Comparative effects of curcumin and an analog of curcumin on alcohol and PUFA induced oxidative stress. *Journal of Pharmacy & Pharmaceutical Sciences*, **7**, 274-283.

Sairam, K., Priyambada, S., Aryya, N.C. and Goel, R.K. (2003) Gastroduodenal ulcer protective activity of *Asparagus racemosus*: an experimental, biochemical and histological study. *Journal of Ethnopharmacology*, **86**, 1-10.

Sairam, K., Rao, C.V., Babu, M.D., Kumar V., Agrawal V.K. and Goel, R.K. (2002) Antiulcerogenic effect of methanolic extract of *Emblica officinalis*: an experimental study. *Journal of Ethnopharmacology*, **82**, 1-9.

Sanmugapriya, E. and Venkataraman, S. (2007) Antiulcerogenic potential of *Strychnos potatorum* Linn seeds on aspirin plus pyloric ligation-induced ulcers in experimental rats.

Phytomedicine, **14**, 360-365.

Sannomiya, M., Cardoso, C.R.P., Figueiredo, M.E., Rodrigues, C.M., dos Santos, L.C., dos Santos, F.V., Serpeloni, J.M., Colus, I.M.S., Vilegas, W. and Varanda, E.A. (2007) Mutagenic evaluation and chemical investigation of *Byrsonima intermedia* A. Juss. Leaf extracts. *Journal of Ethnopharmacology*, **112**, 319-326.

Sannomiya, M., Fonseca, V.B., da Silva, M.A., Rocha, L.R.M., dos Santos, L.C., Hiruma-Lima, C.A., Brito, A.R.M.S. and Vilegas, W. (2005) Flavonoids and antiulcerogenic activity from *Byrsonima crassa* leaves extracts. *Journal of Ethnopharmacology*, **97**, 1-6.

Sener, G., Sehirli, A.O., Gedik, N. and Dulger, G.A. (2007) Rosiglitazone, a PPAR-γ ligand, protects against burn-induced oxidative injury of remote organs. *Burns,* **33**, 587-593.

Shinno. E., Shimoji, M., Imaizumi, N., Kinoshita, S., Sunakawa, H. and Yoko (2005) Activation of rat liver microsomal glutathione *S*-transferase by gallic acid. *Life Sciences*, **78**, 99-106.

Singh, S. and Majumdar, D.K. (1999) Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). *Journal of Ethnopharmacology,* **65**, 13-19.

Sonnenberg, A. (1986) Dietary salt and gastric ulcer. *Gut*, **27**, 1138-1142.

Sridhar, S.B., Sheetal, U.D., Pai, M.R.S.M. and Shastri, M.S. (2005) Preclinical evaluation of the antidiabetic effect of *Eugenia jambolana* seed powder in steptozotocin-diabetic rats. *Brazilian Journal of Medical and Biological Research*, **38**, 463-468.

Sudnikovich, E., Maksimchik, Y.Z., Zabrodskaya, S.V., Kubyshin, V.L., Lapshina, E.A., Bryszewska, M., Reiter, R.J. and Zavodnik, I.B. (2007) Malatonin attenuates metabolic disorders due to streptozotocin-induced diabetes in rats. *European Journal of Pharmacology*, **569**, 180-187.

Thompson, J.H. (1983) Drugs that affect the gastrointestinal system. *In*: Bevan, J.A. and Thompson, J.H. (eds.) *Essentials of pharmacology* 3rd ed. Philadelphia: Harper & Row Publishers. Pp. 384-389.

Tian, Y., Jiang, B., An, L. and Bao, Y. (2007) Neuroprotective effect of catalpol against MPP^+-induced oxidative stress in mesencephalic neurons. *European Journal of Pharmacology*, **568**, 142-148.

Toma, W., Hiruma-Lima, C.A., Guerrero, R.O. and Britod A.R.M.S., (2005) Preliminary studies of *Mammea americana* L. (Guttiferae) bark/latex extract point to an effective antiulcer effect on gastric ulcer models in mice. *Phytomedicine*, **12**, 345-350.

Tortora, G.J. and Derrickson, B. (2006) *Principles of anatomy and physiology* 11th ed. Hoboken USA: John Wiley & Sons Inc. pp. 911-939.

Tripathi, K.D. (2003) *Essentials of medical pharmacology* 5th ed. New Delhi: Jaypee Brothers Medical Publishers. pp. 587-598.

Umamaheswari, M., Asokkumar, K., Rathidevi, R., Sivashanmugam, A.T., Subhadradevi, V. and Ravi, T.K. (2007) Antiulcer and *in vitro* antioxidant activities of *Jasminum grandiflorum* L. *Journal of Ethnopharmacology,* **110**, 464-470.

Veluri, R., Singh, R.P., Liu, Z., Thompson, J.A., Agarwal, R. and Agarwal, C. (2006) Fractionation of grape seed extract and identification of gallic acid as one of the major active constituents causing growth inhibition and apoptotic

death of DU145 human prostate carcinoma cells. *Carcinogenesis*, **27**, 1445-1453.

Venkataranganna, M.V., Gopumadhavan, S. S., Sundaram, R. and Mitra, S.K. (1998) Evaluation of possible mechanism of anti-ulcerogenic activity of UL-409, a herbal preparation. *Journal of Ethnopharmacology*, **63**, 187-192.

Wagner, H. (2006) Multitarget therapy – The future of treatment for more than just functional dyspepsia. *Phytomedicine*, **13**, 122-129.

Walker, V. and Taylor, W.H. (1979) Cigarette smoking, chronic peptic ulceration, and pepsin 1 secretion. *Gut*, **20**, 971-976.

Waugh, A. and Grant, A. (2006) *Ross and Wilson Anatomy and physiology in health and illness* 10th ed. Edinburgh: Churchill Livingstone. pp. 281-334.

Yen, G., Duh, P. and Tsai, H. (2002) Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. *Food Chemistry*, **79**, 307-313.

Yesilada, E., Gurbuz, I. and Ergun, E. (1997) Effects of *Cistus laurifolius* L. flowers on gastric and duodenal lesions. *Journal of Ethnopharmacology*, **55**, 201-211.

Yesilada, E., Takaishi, Y., Fujita, T. and Sezik, E. (2000) Anti-ulcerogenic effects of *Spartium junceum* flowers on *in vivo* test models in rats. *Journal of Ethnopharmacology*, **70**, 219-226.